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ABSTRACTS

The Society for Investigative Dermatology, Inc. and The Japanese Society for Investigative Dermatology Joint International Meeting

The Capital Hilton Hotel
Washington, D.C.
May 1–5, 1985

Wednesday, May 1, 1985

9:00 AM–5:00 PM	Opening Board of Directors Meetings:	
	SID	California
	JSID	Ohio
2:00 PM–8:00 PM	Registration	Upper Lobby
4:00 PM–7:30 PM	Installation of posters for Session I	

7:30–9:30 WORKSHOPS

1. Carcinogenesis David Bickers Tamotsu Kanzaki	Army and Navy
2. Immunocompetent Cells in the Epidermis Gerald Krueger Kiyoshi Nishioka	Congressional
3. AIDS Bijan Safai Volsram Sperry	South American
4. Phototoxicity Masamitsu Ichihashi Kenneth Kraemer	Continental
5. Chemical Mediators John Voorhees Shoso Yamamoto	Senate
6. Pathogenesis of Bullous Diseases Luis Diaz Takeji Nishikawa	Pan American
7. Cell Matrix Richard A. Clark Hideo Yaoita	California
8. ATL and Related Diseases Paul Bunn Shoichi Hatanaka	New York
9. Membrane Glycosylation Miriam Brysk You Kobata	Ohio
10. Melanoma Jean-Claude Bystryn Kowichi Jimbow	Federal
11. Photoimmunology Paul Bergstresser Takeshi Horio	Massachusetts

9:30 PM–10:30 PM	Coffee and Dessert	Presidential Ballroom

Thursday, May 2, 1985

7:30 AM–5:00 PM	Registration	Upper Lobby
7:00 AM–7:30 AM	Continental Breakfast For Residents and Fellows only	South American

IRVIN H. BLANK RESIDENT/FELLOW FORUM
Irwin M. Freedberg, Presiding

Epidermal Differentiation

Introduction	Irwin M. Freedberg, M.D.
Epidermal Stem Cells	Robert Lavker, Ph.D. University of Pennsylvania
Keratin and Keratin Genes	Peter Steinert, Ph.D. National Institutes of Health
Keratohyalin Granules and Filaggrin	Beverly Dale, Ph.D. University of Washington
Involucrin and Keratolinin	Kirk Wuepper, M.D. University of Oregon

8:00 AM–9:00 AM
Business Meetings:
SID—William Epstein, presiding — Presidential Ballroom
JSID—Nobuyuki Mizuno, presiding — Congressional Ballroom

9:00 AM
Welcoming Remarks — Presidential Ballroom
William L. Epstein and Nobuyuki Mizuno

9:30 AM–12:15 PM — Presidential Ballroom

PLENARY SESSION I
D. Martin Carter and Yutaka Mishima, Presiding

1. Characterization of Cloned Suppressor T-Cell Lines that Inhibit the Rejection of Ultraviolet Radiation Induced Tumors. L. K. ROBERTS. University of Utah School of Medicine, Salt Lake City, UT.

Ultraviolet radiation (UV) is a potent carcinogen for the induction of skin tumors. In addition, subcarcinogenic doses of UV induce a population of suppressor T-cells (Ts-cells) that promote the emergence

and growth of UV-tumors. The purpose of this study was to determine whether the functional antigen specificity of the UV-induced Ts-cell population is heterogeneous, consisting of multiple Ts-cell clones able to recognize a range of unique tumor-antigens, or homogeneous, a single Ts-cell clone with functional specificity restricted to a common immunoregulatory tumor-antigen, in nature. Interleukin 2 dependent cloned Ts-cell lines were derived, by limiting dilution without exogenous antigen stimulation, from the splenic T-cell population of a C3H mouse that was exposed to a subcarcinogenic dose of UV. Individual Ts-cell clones were selected for their ability to inhibit the in vitro differentiation of cytotoxic T-cells from the draining lymph nodes of UV-tumor immune mice. When adoptively transferred into non-UV-irradiated syngeneic mice, which normally reject a UV-tumor implant these cloned Ts-cell lines rendered their hosts susceptible to the growth of UV-tumors. These cloned Ts-cells did not inhibit the elicitation of contact hypersensitivity responses, the rejection of allogeneic skin grafts, or the rejection of allogeneic UV-tumors. Employing an in vitro cell binding assay, these cloned Ts-cells were found to bind specifically to UV-tumors. Phenotypically, these cloned Ts-cell lines were identified as Thy 1.2+, Lyt 1+, 2- and I-J+. This study indicates that the UV-induced Ts-cell population is clonal in nature and functions through its ability to recognize common tumor-antigens that are expressed by UV-tumors.

2. Stimulation of *In Vitro* Skin Collagenase Expression by Platelet-derived Growth Factors. E. A. BAUER, T. W. COOPER, J. S. HUANG, J. ALTMAN, AND T. F. DEUEL, Divisions of Dermatology and Hematology-Oncology, Washington University School of Medicine, St. Louis, MO.

Platelet-derived growth factor (PDGF) is postulated to be both chemoattractant and mitogenic for stromal cells in wound healing when blood vessel integrity is compromised and platelet activation occurs. Here, since collagenase is important in wound remodeling, we examined the effects of PDGF on collagenase expression by normal human skin fibroblasts. Culturing cells for 24 hr in the presence of 0–180 ng/ml PDGF resulted in a dose-dependent, saturable increase in collagenase activity in the culture medium that was paralleled by equal increases in immunoreactive collagenase protein, suggesting enhanced synthesis of a catalytically unaltered enzyme. The specificity of this effect was demonstrated by comparing the collagenase-stimulatory effect with that on total protein synthesis and DNA synthesis. Under *in vitro* conditions that produced a 2.5-fold increase in collagenase synthesis, there was an ~20% increase in total protein synthesis and no change in DNA synthesis. In addition, platelet factor 4, another platelet-derived mitogen, caused a <20% increase in collagenase expression. In time course studies, stimulation of collagenase synthesis was first observed 8–10 hr after exposure to PDGF. Conversely, when cells were primed with PDGF for ~24 hr and the stimulator was then removed, an increased rate of synthesis was seen for an additional ~6 hr, after which the rate reverted to control levels. Since the kinetic data suggested a possible pretranslational effect, fibroblasts cultured with PDGF were used to prepare mRNA. In cell-free translation total protein synthesis was essentially unaltered; however, the growth factor caused a >2-fold increase in translatable collagenase mRNA. The data suggest that PDGF specifically modulates collagenase synthesis, possibly through a series of events that lead to increased transcription or preferential translation of collagenase mRNA.

3. Adult T-Cell Leukemia/Lymphoma (ATL): Clinical Diversity and Significance of Detection of HTLV-I Proviral DNA. KIYOSHI TAKATSUKI AND KAZUNARI YAMAGUCHI, The Second Dept. of Internal Medicine, Kumamoto University Medical School, Kumamoto, Japan.

ATL, a unique malignancy first described in Japan, shares some features with Sezary syndrome but is distinct. Variations in the clinical manifestations of atypical cases suggest a division of spectrum of ATL into five types: acute, chronic, smoldering, crisis, and lymphoma. Skin involvement shows various manifestations including generalized erythroderma and localized nodule formation. Biopsy demonstrates the dermal and sometimes epidermal infiltration of leukemic cells. Anti-HTLV-I antibodies in the serum was examined by the indirect immunofluorescence using MT-1 cells as the target cells or the ELISA using disrupted virus particles as the antigens. HTLV-I proviral DNA integrated in the cellular DNA was also examined by Southern blotting in the peripheral blood cells and/or lymphnode cells (in collaboration with Dr. Mitsuaki Yoshida, Cancer Institute, Tokyo). In all patients with ATL, viral antibodies in the serum and proviral DNA in the malignant cells were detected. Since healthy adults are not infrequently positive for viral antibodies in the endemic areas, the detection of proviral DNA provides a powerful tool for understanding the concept of ATL.

4. Chronic Oral Feeding of Ellagic Acid Inhibits 3-Methylcholanthrene-induced Skin Tumorigenesis in Balb/c Mice. MUKUL DAS, DAVID R. BICKERS, AND HASAN MUKHTAR, Dept. of Dermatology, Case Western Reserve University and VA Medical Center, Cleveland, OH.

Polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP) or 3-methylcholanthrene (MCA) induce skin cancer because of their metabolic transformation into highly reactive species which bind to DNA. This led us to the hypothesis that chemical inhibitors of this catalytic process might prove useful as anticarcinogenic agents. Ellagic acid (EA) is a plant phenol found in fruits, nuts and vegetables and is ingested by humans. We have recently shown that topical application of EA to Balb/C mice provides strong protection against MCA-induced skin carcinogenesis. In this study the anticarcinogenic effect of chronic oral feeding, for 120 days, of small amounts (18 μg/mouse/day) of EA in drinking water to Balb/C mice was evaluated. Aryl hydrocarbon hydroxylase, 7-ethoxycoumarin O-deethylase and epoxide hydrolase activities in skin and liver of EA-fed mice were significantly lower than the corresponding activities in control animals. Cytosolic glutathione-S-transferase activities in skin as well as liver of the EA-fed group were 44 and 33% higher than in the controls. The extent of binding of topically applied ^{3}H-benzo(a)pyrene to epidermal DNA was 54% lower in the EA-fed mice as compared to control animals. In further studies the incidence of skin tumors in groups of animals receiving topically applied MCA (1.5 μmol twice weekly for 16 weeks) was evaluated. The animals pretreated with orally administered EA had significantly fewer skin tumors and the latent period prior to onset of tumors was substantially prolonged. These studies indicate that chronic oral feeding of low doses of EA to mice affords substantial protection against MCA-induced skin carcinogenicity and the mechanism of this anticarcinogenic effect of EA may relate to chemical modulation of metabolic activation and inactivation processes.

5. A Monoclonal Antibody Specifically Reactive to Human Langerhans Cell. MARI KASHIHARA, MASAMICHI UEDA, YUJI HORIGUCHI, FUKUMI FURUKAWA, MASAO HANAOAKA, SADAO IMAMURA, Dept. of Dermatology, Faculty of Medicine, and Dept. of Pathology, Institute for Virus Research, Kyoto University, Kyoto, Japan.

Langerhans cells (LC) are the tissue macrophages of the skin. They can be identified by the presence of Birbeck granules (BG) in their cytoplasm with electron microscopy. However precursors and development of LC and their distribution in the skin on various skin disorders are still unknown. LC can be detected in the normal skin using monoclonal antibodies such as OKT6,Na1-43 or V1-CY1. But the specificity of these monoclonal antibodies is not strict.

We attempted to prepare a mouse monoclonal antibody which specifically binds to human LC. The spleen cells from BALB/c mice hyperimmunized with normal human epidermal cells, were hybridized with mouse myeloma cells (X63Ag8.653). Ninety-five out of 283 hybridoma cultures secreted antibodies reactive with frozen sections of normal human skin. One of them secreted a monoclonal antibody which specifically reacted to human LC. Isotype of this antibody (Lag) was IgG_1. In immunoelectron microscopy, Lag was found reactive only to the extramembranous component of BG. With this monoclonal antibody, we could easily identify LC on the frozen tissue sections by light microscopy. Lag-positive cells were found in epidermis, dermis, skin appendages, tongue, esophagus, lymph nodes, thymus and bone marrow. Lag binded only to LC but failed to the other tissue macrophages, monocytes and lymphoid cells. By the affinity chromatography with Lag, proteins whose molecular weights were 39 K, 39–41 K and 64 K, were isolated from the human epidermal sheets.

This antibody offers a new tool for the study of LC biology.

6. Anti-Basement Membrane Zone Antibodies in Bullous Eruption of Systemic Lupus Erythematosus Recognize Epidermolysis Bullosa Acquisita Autoantigen. W. RAY GAMMON, DAVID T. WOODLEY, KENNETH C. DOLE, AND ROBERT A. BRIGGAMAN, Dept. of Dermatology, University of North Carolina School of Medicine, Chapel Hill, NC.

Circulation and tissue-deposited IgG antibodies to the cutaneous basement membrane zone (BMZ) were detected in three patients with

the clinical, pathologic and immunologic features of bullous eruption of systemic lupus erythematosus (SLE). The antibodies were present in sera and IgG fractions in all cases and in eluates of cutaneous immune deposits from one of the cases. The antibodies were easily detected in sera by indirect immunofluorescence on adult human thigh skin separated through the lamina lucida by incubation in 1.0 M NaCl but were less easily detected on intact neonatal foreskin. The antibodies had features of epidermolysis bullosa acquisita (EBA) anti-BMZ antibodies including binding to the dermal side of the BMZ in separated skin, binding to the cutaneous but not vascular or glomerular basement membranes, binding to and just below the lamina densa and binding to 290 or 290 and 145 kd dermal proteins previously identified as components of the EBA autoantigen. The antibodies were relatively specific for SLE patients with features of bullous eruption of SLE since they were detected in 3 of 4 of those cases and in only 1 of 20 SLE patients without blisters. These results show anti-BMZ antibodies are present in patients with features of bullous eruption of SLE and suggest there is a close relationship between that disease and EBA. The results also suggest that EBA antibodies may be part of the autoantibody spectrum of SLE and that separated skin is more sensitive than intact skin for their detection.

7. Con-A Stimulated Thy-1+ Epidermal Cells Exhibit Natural Killer (NK)-like Activity. J. L. NIXON-FULTON, P. R. BERGSTRESSER, J. HACKETT, JR., V. KUMAR, AND R. E. TIGELAAR. Depts. of Dermatology and Pathology, UTHSCD, Dallas, TX.

Mouse epidermal cells (EC) are composed of at least two cell populations which are bone marrow-derived: Ia+ Langerhans cells and an Ia− dendritic cell population which expresses large amounts of Thy-1 antigen (Thy-1 + dEC). Thy-1+dEC lack typical T cell markers such as Lyt-1, Lyt-2, and L3T4; however, they do express asialo GM1 and Ly-5 antigens, in common with NK cells and certain other leukocytes. The purpose of the present study was to determine whether mouse EC enriched for Thy-1+dEC might possess functional activities in common with other Thy-1+ cells and/or NK cells.

Cell suspensions were prepared from trypsin-disaggregated epidermal sheets from CBA mice and enriched to 20–40% Thy-1+dEC by Isolymph separation. These enriched EC, as well as purified Thy-1+dEC, proliferated in short-term cultures in response to Con A; this proliferative response was markedly enhanced by small amounts of IL-2. Long-term (>8 weeks) cultures of EC were established by repeated stimulation with Con A and IL-2. Virtually all the cells from such cultures were Thy-1+ and asialo GM1+. EC grown for >6 weeks in the continuous presence of Con A and IL-2 lysed 14 ± 1% of 51Cr-YAC-1 targets at an effector to target ratio (E:T) of 5:1; similar lysis was observed with poly I:C-stimulated spleen cells. EC grown for 3 weeks with both Con A and IL-2 and then for 3–4 weeks with only IL-2 were even more effective, with 55 ± 13% lysis at an E:T of 5:1.

We conclude that the proliferative response to Con A and IL-2 in long-term cultures of EC which are enriched for Thy-1+dEC includes a population of cells which express a functional property of NK cells.

8. Inhibition of Chemotactic Activity of Psoriasis Leukotactic Factor (PLF), Zymosan Activated Serum (ZAS) and C5a DES-ARG by Ultraviolet Irradiation. KENJI ESAKI AND NOBUYUKI MIZUNO, Dept. of Dermatology, Nagoya City University Medical School, Nagoya, Japan.

UV is effective for psoriasis therapy, but its precise action mechanism is not known. PLF is soluble extract from psoriatic scale. It has chemotactic activity, and is certified anaphylatoxin C5a. It is thought to play some role in the formation of psoriatic lesion, even if it is not a causative factor. Accordingly, the effect of UV on chemotactic activity of C5a was investigated in vitro, using PLF, ZAS (Vallota et al.) and C5a des-arg (Upjohn Co.).

Chemotaxis test was examined by modified Boyden's method (Snyderman and Pike). Chemotactic activity of PLF to polymorphonuclear leukocyte (PMN) was inhibited by UVB irradiation ($1.2 \times 10^4 J/m^2$), but not by UVA. Chemotactic activity of ZAS was inhibited by UVB irradiation ($1.2 \times 10^4 J/m^2$). The action spectrum for inactivation of ZAS existed in the range from 265 to 295 nm with the maximum at 280 nm. Although activity of C5a des-arg was not inhibited by UV, its mixture with normal human serum to which 1M EACA was added, was inactivated by UVB. UVB irradiation under anaerobic condition or aerobic condition with 0.05M NaN_3 failed to inactivate ZAS. Chemotactic activity of casein and E. coli culture filtrate was not inhibited by UVB even by mixing with normal human serum. Therefore the production of leukotactic inhibitor in normal serum by UV can be excluded.

These results indicate that singlet oxygen is primarily formed by photoactivation of some chromophore in serum, and C5a is inactivated secondarily by a chemical process involving singlet oxygen. Inactivation of C5a in psoriatic scale by UV seems to be one of the action mechanisms for psoriasis therapy.

9. A Monoclonal Antibody Against Desmoglein I (DGI) Binds to Pemphigus Foliaceus (PF) Antigen. J. R. STANLEY, L. KOULU, V. KLAUS-KOVTUN, AND M. S. STEINBERG, Uniformed Services University of the Health Sciences, Bethesda, MD, and Princeton University, Princeton, NJ.

PF is an autoimmune antibody-mediated disease in which acantholysis occurs in the superficial epidermis. Electron microscopy of early lesions has demonstrated abnormalities in the desmosome-tonofilament complex. We have previously demonstrated that autoantibodies from about half of PF patients specifically bind a 160-kD protein ("PF antigen") extracted from normal human epidermis. DGI is an approximately 160-kD glycoprotein isolated from the core of bovine muzzle desmosomes. In this study we demonstrate that a monoclonal antibody, 3D3, raised against DGI also binds to PF antigen. The specificity of 3D3 was demonstrated by immunoblot analysis of bovine muzzle desmosomal proteins separated by SDS-PAGE. 3D3 stained only DGI. Immunofluorescence, using double labeling with rhodamine-goat-anti-mouse IgG and fluorescein-goat-anti-human IgG, demonstrated that 3D3 and PF antibodies bind in identical patterns on the same section of normal human skin. To determine whether 3D3 and PF autoantibodies bind the same polypeptide, we used immunoblot analysis of normal human epidermal extracts. One-dimensional SDS-PAGE indicated that PF antibodies from 7 of 15 patients bind a 160-kD broad band. 3D3 binds a comigrating band. Pemphigus vulgaris and normal human sera do not bind this band. Two-dimensional electrophoresis (isoelectric focusing followed by SDS-PAGE) indicated that 3D3 and PF autoantibodies bind the same spots, with pI approximately 5.7. These studies indicate that certain patients with PF have autoantibodies directed against DGI, a desmosomal core glycoprotein.

10. Immunological Relationship Between Vitiligo and Melanoma. G. K. NAUGHTON AND J-C. BYSTRYN, Dept. of Dermatology, NYU School of Medicine, New York, NY.

A number of clinical observations suggest there is a link between vitiligo and melanoma. Because the cause of this link is not known, we conducted the following experiments to examine whether there is a relationship in immune responses to pigmented cells in the two diseases. Sera of 52 individuals with vitiligo, 46 with melanoma, and 35 normal individuals were tested for antibodies to human melanoma and control cells using a protein A-sepharose immunoprecipitation assay. The antigen source was detergent lysate of radioiodinated cells. Antibodies to surface antigens on pigmented melanoma cells were found in 48 (92%) persons with vitiligo, 17 (37%) of those with melanoma, and in 3 (8.5%) of normals. By SDS-PAGE analysis, the antibodies in patients with vitiligo all reacted with a surface protein of 85 kds as well as to two other proteins with MWs of approximately 75 and 250 kds. The antibodies in some of the patients with melanoma also reacted with an 85 kd surface protein. By sequential immunodepletion, the 85 kd antigen defined by vitiligo and melanoma antibodies appeared to be similar. This antigen was expressed by pigmented melanoma and normal melanocytes but not by nonpigmented melanomas or other control cells.

Thus, patients with vitiligo usually and those with melanoma occasionally develop an immune response to a surface antigen which is shared by, and selectively expressed on, melanocytes and pigmented melanoma cells. This observation suggests that the link between vitiligo and melanoma results from immune responses directed to antigens shared by normal and malignant pigmented cells.

11. Cure of Malignant Melanoma by Selective Atomic Reactor Therapy Using Melanoma-Seeking Compounds: ^{10}B/Melanogenesis Interaction from In Vitro/In Vivo Radiobiological Analysis to Preclinical Studies. YUTAKA MISHIMA, MASAMITSU ICHIHASHI, MASAYUKI TSUJI, MASATO UEDA, SUSUMU HATTA, AND TATSUO SUZUKI*, Dept.of Dermatology, Kobe University School of Medicine, Kobe, Japan, and * School of Veterinary Medicine, Azabu University, Sagamihara, Japan.

Effectiveness of high LET particle irradiation is limited by the inability to localize its absorbed energy to cancer cells at the cellular level. Thermal neutrons are easily absorbed by the non-radioactive isotope ^{10}B, resulting in the emission of α-particles and lithium atoms

which release energy of 2.33 MeV up to 14μ, the diameter of melanoma cells. Thus if we selectively accumulate ^{10}B in melanoma, we can destroy it without injury to the surrounding normal tissues by concentrating high LET particles. Melanoma usually accentuates melanin synthesis. We therefore synthesized ^{10}B melanin substrate analogues, of which $^{10}B_1$-p-boronophenylalanine ($^{10}B_1$-BPA) is most effective. In vitro radiobiological analysis reveals the highly enhanced melanoma killing effect of $^{10}B_1$-BPA. The D_0 value of neutron only is 2.8×10^{12} nvt but $^{10}B_1$-BPA, 0.38μg ^{10}B/ml pre-incubation, 0.9×10^{12} nvt. Chemical analysis of melanoma bearing hamsters 28 hrs after systemic $^{10}B_1$-BPA reveals a ^{10}B tumor/blood ratio of 11.5 and a tumor/liver 15. We then irradiated these hamsters and found that the maximal suppression was obtained with neutron irradiation following systemic $^{10}B_1$-BPA, as compared to neutron or X-ray alone. We advanced to pre-clinical studies using spontaneously occurring melanoma in Duroc pig skin. We cured three melanoma cases, 4.6 to 12 cm in diameter, by single treatment of neutron capture when 5 mg $^{10}B_1$-BPA was injected perilesionally prior to irradiation of $1.3 \sim 2.6 \times 10^{13}$n/cm^2. Complete disappearance of melanoma was obtained without substantial side effects. In a control study, cure of one half a melanoma was obtained when irradiation was limited to that side only.

12. Dexamethasone Inhibits Plasminogen Activation But Does Not Block Pemphigus IgG Induced Acantholysis *In Vivo*. G. J. ANHALT, H. P. PATEL, R. S. LABIB, L. A. DIAZ, AND D. PROUD, Depts. of Dermatology and Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD.

An increase in plasminogen activator (PA) activity has been implicated in the pathogenesis of pemphigus vulgaris (PV). Our laboratory has shown that passive transfer of human PV autoantibodies (PV IgG) in neonatal BALB/c mice can reproduce the disease. We studied the levels of PA activity in mouse skin after intraperitoneal (i.p.) injection of normal human IgG (NH IgG) and PV IgG, with and without exposure to Dexamethasone (Dex). All animals received i.p. injections of saline control or Dex in a dose of 20 mg/kg body weight. 24 hours later, they received identical injections of saline or Dex and a single dose of NH or PV IgG (10 mg/gm body weight). The next day, all animals were graded for extent of clinical disease, and the whole skin homogenized in 0.5% Triton X-100 in 0.1M Tris at 4°C. The supernatants were assayed for PA activity by a two stage colorimetric reaction and plasmin activity was measured by cleavage of thiobenzyl benzyloxycarbonyl L-lysinate and expressed as milli Ploug units per mg. protein. In those animals injected with NH IgG, PA activity was 4.48 ± 0.85 ($n = 10$), and was reduced to 2.68 ± 0.37 ($n = 10$) with Dex treatment ($p < .001$). Animals injected with PV IgG had PA activities of 2.97 ± 0.96 ($n = 9$) and was reduced to 1.24 ± 0.24 ($n = 10$) with Dex treatment ($p < .001$). Nonspecific proteinase activity was similar in all groups. Despite the decreased PA activity, all animals in the PV IgG and the PV IgG plus Dex group had identical clinical disease with a positive Nikolsky sign and extensive erosions, and lesions developed at the same time points. The role of PA activity in the genesis of cutaneous lesions of PV *in vivo* requires further definition.

13. Active Psoriatic Lesions Contain Increased Levels of Epidermal Growth Factor (EGF) Receptors. L. B. NANNEY, CHRISTA STOSCHECK, AND L. E. KING, JR., Depts. of Plastic Surgery and Anatomy, and Medicine (Dermatology), Vanderbilt and V.A. Medical Centers, Nashville, TN.

EGF stimulates both growth and differentiation in normal human epithelial cells, but its effects on hyperproliferative skin diseases is unknown. EGF metabolism in psoriasis vulgaris was therefore studied morphologically (by autoradiography and immunocytochemistry using anti-EGF receptor antibody) and biochemically (^{125}I-EGF binding, EGF stimulated tyrosine kinase). EGF binding could not be detected in whole 3mm punch biopsies of normal or psoriatic adult skin. However, the human epidermis isolated by dispase treatment contained 2.52 pg EGF bound per microgram of cellular membranes. EGF stimulated phosphorylation was observed in membranes obtained from human epidermis isolated by scraping frozen skin or in 3mm punch biopsies. Electrophoresis revealed that the major EGF sensitive phosphate acceptor migrated as a 170,000 molecular weight protein. Since this is the molecular weight of the EGF receptor, we tentatively identify the phosphorylated band to be the epidermal EGF receptor. In normal human epidermis, the germinative layer contained 4-fold more EGF receptors than the upper epidermal layers as measured by immunocytochemistry (JID 82:169, 1983) or autoradiography (16.5 ± 1.7 vs 4.1 ± 0.8 silver grains/μM^2, respectively.) In active psoriatic lesions, the germinative layer contained approximately the same number of EGF receptors (14.7 ± 2.8 silver grains/μM^2) as normal epidermis, but the upper layers contained twice as many (8.8 ± 0.7) EGF receptors. Thus the major aberration of EGF receptor metabolism in psoriasis is its persistence in the upper nonproliferating cell layers, rather than a general increase throughout the epidermis or an increase in the dividing cell population.

Conclusion: The persistence of increased EGF receptors in the upper but not the basal epidermal layers indicates that the EGF abnormalities may have characteristics of changes in differentiation other than hyperproliferation.

1:30 PM–2:15 PM Presidential Ballroom

JSID GUEST SPEAKER

Nobuyuki Mizuno, Presiding

Soichi Hatanaka

"Adult T-Cell Leukemia/Lymphoma: Recent Progress in Japan"

2:30 PM–5:30 PM

CONCURRENT SCIENTIFIC SESSIONS

CONCURRENT SESSION 1A: CELLULAR IMMUNOLOGY

Presidential Ballroom

Neil Brody and Hiroaki Ueki, Presiding

1. I-A Restricted T Cell Proliferative Responses to Sulbenicillin (SBPC) in Mice. ZENRO IKEZAWA, HIROSHI KAWAGUCHI, MASAHIRO SATO, AND RYUKICHI NAGAI, Dept. of Dermatology, Yokohoma City University School of Medicine, Yokohama, Japan.

Few investigations have been made on the genetic control of drug hypersensitivity. As a first step in studying the genetic control of penicillin hypersensitivity, which is very common in man, semisynthetic pencillin, sulbenicillin (SBPC) was used to induce TC cell proliferation in mice. The lymph node cells taken from the mice immunized with 10mg SBPC in complete Freund's adjuvant, were tested for antigen-specific response in a standard 3 days proliferation assay. The Lyt.1$^+$2$^-$ cell proliferative responsiveness to SBPC was mapped to the I-A locus in H-2 major histocompatibility complex (MHC). Also the proliferation was inhibited by the Aβ-specific monoclonal antibodies, but was not by the Eα-specific monoclonal antibodies. These results suggested that the T cell responses to SBPC were controlled by the I-A locus in the MHC, the products of which, that is the MHC class II A molecules, function as restriction molecules in T cell recognition of SBPC. This is the first report indicating the MHC control of the T cell responses to penicillin in mice.

2. Generation of Hapten- and Epidermal-specific T Cell Clones. S. SHIMADA AND S. I. KATZ, Dermatology Branch, NCI, NIH, Bethesda, MD.

The most effective method for the induction of hapten-specific allergic contact dermatitis (ACD) is via epicutaneous application of the hapten. Another effective method is by the administration of haptenated epidermal cells (EC) subcutaneously (sq). The latter method induces more intense and longer lasting ACD than does the sq administration of haptenated spleen cells (SC). Thus there may be something unique about EC which, when haptenated, allows them to generate effector cells more effectively than do SC. We therefore attempted to generate hapten and epidermal-specific T cell clones. 4 days after painting mice with 7% trinitrochlorobenzene, draining lymph node cells were obtained and T cells purified. These cells were co-cultured with trinitrophenylated (TNP) Langerhans cell-enriched EC. After 4 days, cells were harvested and rested on non-TNP conjugated EC. The cells were restimulated and rested three times and cloned by limiting dilution with added Interleukin 2 which was then continually added. Proliferation of T cells was assessed by ^{3}H-thymidine incorporation. Cytotoxicity assays utilized TNP-conjugated Concanavalin A SC blasts or EC as targets. Clones A-2 and E-4 are Thy-1$^+$, Ly 2$^+$and L3T4$^-$ and TNP-specific. In contrast to noncloned TNP-specific T cells, the clones proliferate preferentially in response to TNP-EC rather than TNP-SC. Also in contrast to noncloned T cells, the clones were preferentially cytotoxic for TNP-EC; there was an 8 to 32 fold increase in killing TNP-EC rather than TNP-SC. Clones A-2 and E-4 therefore exhibit hapten and epidermal specificity. The epidermal specific epitope which

is recognized is unknown but genetic restriction and antibody inhibition studies indicate that it is co-recognized with H-2K.

3. Dynamic Change of the Distribution of Epidermal Ia Antigen in Allergic Contact Sensitivity Reaction in Mice. S. AIBA AND H. TAGAMI, Dept. of Dermatology, Tohoku University School of Medicine, Sendai, Japan.

Recently we described the presence of two different stages in regard to distribution pattern of the epidermal Ia antigens in allergic contact sensitivity reactions of mice (Brit. J. Dermatol. in press). The first stage was noted until 3 days post-challenge, when epidermal Ia-positive cells were composed only of dendritic Langerhans cells (LCs); their number was decreased, whereas their size enlarged significantly with extending dendrites. Thereafter keratinocytes began to express Ia antigens. Since these data were obtained only from the immunohistologic observation of EDTA-separated epidermal sheets, we still lack quantitative information about the precise amount of Ia antigen expressed in such epidermis as well as actual number of Ia positive cells. Therefore the change of the amount of Ia antigen on the surface of LCs and the number of the Ia antigen positive cells were analyzed by fluorescence activated cell sorter. The epidermal cell suspensions were made from the ears of TNCB sensitized mice by the treatment of 0.25% trypsin and 0.05% DNase at various time intervals after TNCB challenge and were stained with anti-Ia monoclonal antibody and FITC-labelled anti-mouse immunoglobulin antibody. The percentage of Ia positive cells changed from 6% at 0 hr to 24% at 24 hr, 32% at 72 hr, and 30% at 120 hr post challenge. An increase in the amount of Ia antigen on the cell surface was also recognized in such skin. These data now provide a definite quantitative basis for the occurrence of a dynamic change of Ia antigen distribution in the epidermis of allergic contact sensitivity reactions, in which keratincytes begin to express cell surface Ia antigen even from 24 hrs postchallenge, much earlier than we have thought.

4. Alloantigen-induced Delayed Type Hypersensitivity Using Separate Epidermal Cells. NORIHISA ISHII, HIROSHI KAWAGUCHI, HIROSHI NAKAJIMA, AND RYUKICHI NAGAI, Dept. of Dermatology, Yokohama City University School of Medicine, Yokohama, Japan.

The mechanism of allograft rejection involved in cell mediated process was studied. In the present study we invented alloantigen-mediated delayed type hypersensitivity (DTH) responses using trypsin-treated separate epidermal cells (ECs). A lot of congenic strains of mice were used to study the mechanism of these alloantigen-mediated DTH responses. Mice were immunized with separate ECs subcutaneously. Seven days later, these mice were challenged into hind footpads with ECs. DTH response was assayed by footpad swellings. The first experiments were performed to determine the importance of both H-2 and non-H-2 antigens to elicit DTH response in mice. Our data revealed that difference in either H-2 or non-H-2 antigens between donor and recipient mice were sufficient to elicit a DTH response. Since the elicitation of footpad swellings was seen in any allogeneic combination, the donor ECs were treated various monoclonal anti H-2 antibodies plus complement. Interestingly, the treatment of anti I-A, I-E, or K antibody plus complement could eliminate the strong alloantigen-mediated DTH responses. These results suggest that cells with I-A, I-E, or K region on their cell surfaces might be playing some important roles in these alloantigen-mediated DTH responses. Transfer experiments were carried out to study what kind of cells were important to transfer these DTH responses. T cells sensitized by ECs successfully transferred DTH responses into naive mice. Further studies revealed that these cells were Lyt-1^+2^-, Ia^- cells, since treatment of these T cells with monoclonal antibodies plus complement abolished the ability of transfer. Our present data clearly demonstrate that these separate ECs could induce strong alloantigen-mediated DTH responses.

5. Induction and Suppression of Contact Sensitivity by Liposomes Bearing Hapten-conjugated Molecules from Epidermal Cells. K. NISHIOKA, H. YOKOZEKI, I. KATAYAMA, C. TAKIJIRI, AND K. HASHIMOTO, Dept. of Dermatology, Osaka University School of Medicine, Osaka, Japan.

Liposomes carrying molecules from hapten-conjugated epidermal cells stimulated the sensitized lymphocytes effectively (JID 83; 96, 1984). The aim of this study is to examine whether the liposome can sensitize animals in vivo. Liposomes were prepared with egg lecithin, cholesterol and octylglucoside. Deoxycholate-extract of TNP-epidermal cells was incorporated into the liposome. The liposome was injected subcutaneously into nonsensitized mice. More than 100 μg of the liposome sensitized contact sensitivity in the recipients. This contact sensitivity could be transferred by T cells. An intravenous injection of the liposome induced unresponsiveness of contact sensitivity although an intraperitoneal injection of the liposome induced contact sensitivity. Epidermal cells were treated with anti-Thy 1 and complement to deplete Ia positive population. The treated cells were trinitrophenylated, extracted and incorporated into liposomes. A subcutaneous injection of 100 μg of thus prepared liposomes induced unresponsiveness. And this unresponsiveness was transferred by suppressor T cells. Therefore, it is suggested that Ia antigens in epidermal cells play a critical role in induction and suppression of contact sensitivity.

6. A Comparison of the Nature and Kinetics of Association of a Contact Sensitising and a Non-sensitising Derivative of Nitrobenzene with Cutaneous Antigen-presenting Cells. PHILIP A. BOTHAM AND NICOLA J. RATTRAY, Central Toxicology Laboratory, ICI PLC, Alderley Park, Cheshire, UK.

The role of cutaneous antigen presenting cells in the discrimination between contact sensitising and non-sensitising haptens has been investigated using the Balb/c mouse.

We have utilised two derivatives of nitrobenzene; 2,4-dinitrochlorobenzene (DNCB, a potent sensitiser) and 2,4-dichloronitrobenzene (DCNB, a non-sensitiser). Using monoclonal antibodies specific for the derivatives and for the Ia antigen in double labelling immunofluorescence techniques we have examined the nature and kinetics of association of the chemicals with cutaneous antigen-presenting cells.

Four hours after the application of a sensitising dose of DNCB the chemical had become localised on the membranes of many epidermal cells and around the hair follicles. DNCB could not be detected in the dermis or the lymph nodes. After 24 and 48 hours Ia^+-cells carrying DNCB on their cell surfaces could be seen in the dermis and in the paracortical region of the lymph nodes. By 72 hours no DNCB positive cells could be seen in the dermis, but they were abundant in both the paracortical and medullary regions of the nodes. In contrast, although DCNB also localised within the epidermis after 4 hours, we could not detect either cellular associated or free DCNB in the dermis or the draining lymph nodes up to 72 hours after application.

Although this may reflect differences in other properties of the chemicals (eg protein reactivity) our observations suggest that the capacity of a chemical to induce sensitisation may depend on its ability to stimulate the movement of antigen-presenting cells to the draining nodes.

7. Failure to Elicit Cross-tolerance to 2,4-Dinitrochlorobenzene by Prior Exposure to 2,4-Dinitrothiocyanobenzene. I. KIMBER AND J. MITCHELL, Central Toxicology Laboratory, ICI PLC, Alderley Park, Cheshire, UK.

The induction and role of immunological tolerance in the elicitation of contact sensitivity has been investigated in BALB/c mice.

There now exists compelling evidence that the epicutaneous application of a sensitising chemical results in the induction of hapten-specific helper and suppressor T lymphocytes and that certain non-sensitising chemicals may selectively induce suppressor cells resulting in hyporeactivity.

It has been previously reported that epicutaneous exposure to 2,4-dinithrothiocyanobenzene (DNTB) induces a T cell mediated hyperresponsiveness to sensitising dinitrobenzene analogues. Using similar experimental protocols we have examined the capacity of DNTB to induce tolerance to subsequent 2,4-dinitrocholorobenzene (DNCB) exposure. In contrast to other studies, however, we have demonstrated that DNTB fails to influence responsiveness to DNCB and is in fact a potent sensitiser. These data indicate that the induction of cross-tolerance is not an absolute property of a chemical and that a variety of factors may influence relative immunogenicity. The important variables are as yet unknown although our studies indicate that a rapid T cell-mediated hyposensitisation to DNCB can be demonstrated in BALB/c mice following iv administration of 2,4-dinitrobenzene-1-sulphonic acid sodium salt (750 mg/kg) suggesting the absence of an inherent suppressor cell defect in this strain of animals.

8. Effect of Cyclosporine A on the Induction and Elicitation of Murine Allergic Contact Dermatitis. D. V. BELSITO, B. XUE*, L. POSSICK, R. M. DERSARKISSIAN, R. L. BAER, AND G. D. THORBECKE*, Depts. of Dermatology and * Pathology, New York University School of Medicine, New York, NY.

Cyclosporine A (CY), 75mg/kg, was injected subcutaneously into

groups of mice at varying times during the induction of sensitization or elicitation of allergic contact dermatitis to dinitrofluorobenzene (DNFB). At this dosage, CY appeared to inhibit both the afferent and efferent limbs of the allergic reaction. Mice treated with CY during the time of attempted DNFB sensitization did not develop a long lasting immunotolerance to DNFB. Within 48 hrs. of stopping CY injections, sensitization to DNFB could be accomplished, thus making it unlikely that T suppressor cells were responsible for the lack of contact sensitization. Although CY at this dosage caused an 80–90% reduction in splenic antibody forming cells to Trinitrophenylated Ficoll, 1 μg, intraperitoneally or intravenously, there was no effect on the number of Ia^+, Fc $Receptor^+$ and Common $Leukocyte\ Antigen^+$ epidermal Langerhans cells, even after 10 days of continuous CY-treatment. We postulate that CY inhibits the induction and elicitation of contact allergic reactions by blocking the clonal expansion of T cells, probably secondary to its effect on interleukin-2 production. Studies testing this hypothesis are underway.

9. Concanavalin A (Con A)-induced Proliferation of Mouse Epidermal Cells. R. E. TIGELAAR, J. L. NIXON-FULTON, AND P. R. BERGSTRESSER, Dept. of Dermatology and Internal Medicine, UTHSCD, Dallas, TX.

Mouse eidermal cells (EC) are composed of at least 3 cell types: keratinocytes, Ia^+ Langerhans cells, and dendritic, bone marrow-derived Ia- cells which express Thy-1 antigen (Thy-1 + dEC). Thy-1 + dEC do not express easily detectable amounts of other T cell antigens such as Lyt-1, Lyt-2, or L3T4, and to date, little is known of their functional capabilities. To determine whether Thy-1 + dEC and/or other EC could function analogously to T cells *in vitro*, proliferation assays with the mitogen Con A were performed. Sterile cell suspensions prepared from trypsin-disaggregated sheets of body wall epidermis from CBA mice were enriched to 8–18% Ia^+ and 20–40% $Thy\text{-}1^+$ cells by centrifugation over Isolymph. These EC were then cultured + or − Con A in 96-well U-plates for 2–10 days; proliferation was assessed by quantitating 3H-thymidine incorporated during the final 18 hr of culture. Con A-induced proliferation of EC was readily seen and was proportional to the number of EC/well; as few as 10,000/well gave easily detectable responses with stimulation indices > 10. Optimal stimulation was seen with 2.5 μg/ml Con A at 5 days. Con A responses were enhanced 2x by the continuous presence of 1 μg/ml indomethacin. Responses both in the presence and absence of Con A were markedly enhanced (from 2x to > 20x) in a dose-related way by the addition of small amounts of partially purified rat T cell growth factor (IL-2). Responses in wells stimulated by both Con A and IL-2 continued to increase throughout the 10 day culture period. We conclude that mouse epidermis contains cells which can proliferate *in vitro* in a manner indistinguishable from some T lymphocytes and that the response to IL-2 without Con A may indicate the presence of pre-activated cells.

10. Comparative Cytotoxicity of Human Melanocytes, Keratinocytes, Endothelial Cells and Fibroblasts. L. R. CAPIN, M. G. TONNESSEN, R. L. OSBORN, M. KISSINGER, AND D. A. NORRIS, Dept. of Dermatology, University of Colorado and VA Medical Center, Denver, CO.

Cytotoxicity is an important mechanism in the pathogenesis of many skin diseases. Differential susceptibility of cellular targets to injury may determine, in part, patterns of cutaneous damage. We have previously shown significant advantages of dye techniques over radioactive label techniques in study of human keratinocyte lysis *in vitro*. Here, we report a comparison of 3 different dye techniques to study cellular injury *in vitro* of human newborn foreskin melanocytes (M), keratinocytes (K), and fibroblasts (F) and human microvascular endothelial cells (EC) grown to Lab Tek chamber slides. Increasing concentrations (10^{-7} to 10^{-2} M) of hydrogen peroxide, a known mediator of cytotoxicity, were used to induce cell damage after 2, 3 and 4 hrs. incubation at 37°C. Cell injury was assessed by fluorescein diacetate uptake, by nigrosin exclusion, and by acridine orange uptake/ethidium bromide exclusion. Numbers of injured and viable cells were determined by visual examination of coded specimens, and LD50 values were obtained by probit analysis of the data. Peroxide produced dose and time dependent injury of all 4 types of cutaneous cellular targets. Differential susceptibility to injury was evident: M > EC > K > F.

All 3 dye techniques have distinct advantages in studying these cutaneous cellular targets: 1) monolayers or colonies of cells can be easily counted directly or from photomicrographs, 2) *individual* cell damage can be determined, in distinction to *population* damage assessed by radioactive release assays, 3) small numbers of cells can be evaluated, 4) and a concentration-dependent effect can be seen with LD50 analysis. Such sensitive and reproducible *in vitro* techniques to investigate injury of different human cutaneous cells in culture will be useful in elucidating mechanisms of injury of specific cellular targets.

11. Passive Sensitization of Human Cutaneous Mast Cells in Suspension with Mouse Monoclonal IgE. M. D. THARP, M. C. RAY, M. B. CHAKER, AND T. J. SULLIVAN, Depts. of Dermatology and Allergy, UTHSCD, Dallas, TX.

The ability of human and murine IgE to sensitize suspensions of human cutaneous mast cells was investigated. Following enzymatic digestion of infant skin, 250,000 to 550,000 mast cells were recovered/gm wet weight tissue (1–5% of the total cells). Mast cell viability at 24 hrs was 85.6% (±1.6 SEM) and histamine content was 4.1 pg (±0.8 SEM)/cell. Skin mast cells were characterized by phase contrast, FITC-avidin, metachromatic staining, and electron microscopy which demonstrated normal mast cell morphology. After passive sensitization with human IgE, skin mast cells were stimulated with a mouse monoclonal anti-human IgE which resulted in a net histamine release of 18.4% (± 2.1 SEM). Mast cells sensitized with mouse monoclonal IgE to DNP or to arsenate released histamine (22–52%) following an antigen-specific stimulus. Suspensions of mast cells were incubated either with mouse IgE anti-DNP or with human IgE followed by mouse IgE anti-DNP. Cells incubated with only mouse IgE released histamine following DNP-BSA challenge while cells preincubated sequentially with human and mouse IgE failed to respond to this antigen. These studies indicate that human and mouse of IgE compete for the same functional mast cell IgE receptor. Stimulation of mast cell suspensions with human C3a, calcium ionophore A23187, and morphine also induced significant histamine release; no response to bradykinin was detected. These results indicate that human skin mast cells obtained by enzymatic digestion express functional receptors for IgE, C3a, and morphine, and that human and mouse IgE compete for the same IgE receptor on the human skin mast cell. This new *in vitro* system would be useful for future studies of human mast cell function.

12. Spontaneous Antibodies to Basal Cell in Nude Mice. FUKUMI FURUKAWA AND SADAO IMAMURA, Depts. of Pathology and Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Antibodies to basal cell (BC) of skin have been reported in burn toxemia, bone marrow transplantation and in drug-induced skin eruption. However, the biological significance is still unknown. Recently, we found that spontaneous anti-BC antibodies in nude mice and studied the mode of the appearance of anti-BC antibodies in thymic chimeras.

Indirect immunofluorescence (IF) studies revealed that anti-BC antibodies in 1:10 diluted sera were found in 20% (4/20) of 2-mo-old BALB/c nu/nu mice and in 0% (0/15) of 2-mo BALB/c +/+ mice. Anti-BC antibodies showed positive reaction to BC of skin, lip and esophagus, but not to kidney and liver. As next step, BALB/c nu/nu mice were engrafted with thymus of newborn C57BL/6J (B6) mice and BALB/c +/+ mice. Sixty days after thymus grafts, nude mice were sacrificed. Complete restoration of thymic function in spleen cells could be determined by the number of Thy 1.2 positive cells and by responsiveness to mitogens (PHA, Con A) stimulation. Alloreactivity was tested in assays of mixed lymphocytes culture (MCL) and cytotoxic T lymphocytes (CTL). CTL revealed that nude spleen cells with B6 thymus were completely tolerant to both host and donor-type H-2, but MLC showed tolerance only to host-type H-2. Serological tests showed the presence of anti-BC antibodies in nude mice engrafted with non-treated thymus or irradiated (1,000 rads) thymuses of B6 mice. The incidence was 30% (6/20) and 20% (3/15), respectively. However, nude mice engrafted with syngeneic BALB/c +/+ thymus had no antibodies (0/15).

These results suggest that H-2 restriction in T-B cell interaction plays a significant role in the appearance of these antibodies.

13. Immunophenotyping of Skin Cells Following Epidermal Injury. MICHAEL REUSCH, B. J. NICKOLOFF, J. N. MANSBRIDGE, AND V. B. MORHENN, Psoriasis Research Institute and Dept. of Dermatology, Stanford University School of Medicine, Stanford, CA.

The time course of appearance and the characterization of immunocompetent cells following sterile suction blister wound healing was assessed in four healthy human subjects. Biopsies were obtained at intervals up to 28 days after injury and were stained utilizing an indirect immunoperoxidase technique with monoclonal antibodies against Leu 1, 2a, 3a & b, 4, HLA-DR and OKT6. In general, re-epithelialization of the wound began at day 2 and was completed by day 8. The epidermis

and hair follicles adjacent to the wound contained an increased number (2x) of OKT6$^+$, HLA-DR$^+$, dendritic Langerhans cells (LCs) by day 3–4 reaching a maximum by day 10. Later, LCs appeared in the newly-formed epidermis. An exclusively mononuclear, patchy, perivascular infiltrate appeared at day 1, which subsequently became increasingly dense and oriented along the dermo-epidermal junction and the hair follicles. This infiltrate primarily consisted of T cells which until day 4 were exclusively of the T helper subtype. On days 10 and 28, an increasing proportion (up to 20%) of cytotoxic/suppressor T cells appeared. Paralleling the increase of LCs in the epidermis, the number of OKT6$^+$ cells in the dermis progressively rose. In the mid-dermis, around the vasculature, the OKT6$^+$ cells were predominantly round; in the papillary dermis they were mostly dendritic. They seemed to enter the epidermis with some preference for the hair follicles beneath the wound.

The marked increase in LCs, recruited from the OKT6$^+$ dermal infiltrate, and the appearance and changes in T cell subtypes may be understood as an augmentation of immunological survey at this locus minoris resistentiae. Since lymphocyte-released gamma interferon can inhibit proliferation of keratinocyte cultures, it is conceivable that the immune system also plays a role in regulating epidermal regeneration.

14. Immune Functions of Human Skin Microvascular Endothelial Cells. M. KARASEK, C. CLAYBERGER, AND A. KRENSKY, Depts. of Dermatology and Pediatrics, Stanford University School of Medicine, Stanford, CA.

Cytotoxic T lymphocytes (CTL) and skin microvascular endothelial cells (EN) play central roles in cell mediated immune reactions of skin including graft rejection and tumor killing. To determine the mechanism of these responses, we have studied the interactions of adult T lymphocytes and human skin microvascular endothelial cells in vitro.

CTL were generated by cocultivation of peripheral blood by lymphocytes (PBL) with monolayer cultures of an irradiated (1200 R) newborn endothelial cell line (in passages 3–6). PBL were restimulated at 2 week intervals with irradiated EN, and at 3 day intervals with medium supplemented with interleukin II. Early and late stages of cytolysis were studied by phase contrast microscopy and release of ^{51}Cr.

When CTL were incubated with EN lines from 4 newborn and 2 adult donors, all EN were lysed. In addition, these CTL lysed a variety of human B and T cell tumor lines but did not lyse syngeneic cells. Although these CTL express both OKT3 and OKT8, monoclonal antibodies specific for these molecules as well as monoclonal antibodies specific for Class I and Class II antigens, and beta$_2$-microglobulin did not block lysis. Monoclonal antibodies specific for lymphocyte associated antigens LFA-1 and LFA-3 inhibited cytolysis of B lymphoblast targets but not EN.

These results describe a different mechanism for cytolysis of skin microvascular endothelial cells which does not utilize the same cell surface molecules reported for other nonvascular cell types.

15. Changes in Lymphocyte and Langerhans Cell Populations in Human Irritant Contact Dermatitis. JUDITH V. WILLIAMS, RICHARD J. ZAINO, AND JAMES G. MARKS, JR., Div. of Dermatology, Dept. of Medicine, and the Dept. of Pathology, The Milton S. Hershey Medical Center of the Pennsylvania State University, College of Medicine, Hershey, PA.

The purpose of this study was to examine the changes in lymphoctye and Langerhans cell populations in irritant contact dermatitis. Three volunteers with no previous or current dermatitis were selected for the study. One patch was removed at 24 hours and the patch test site biopsied. The remaining patches were removed at 48 hours and the patch test sites biopsied on days 2, 4, 7, 14 and 21. Monoclonal antibodies directed against (Leu 4) pan T cell, (Leu 3A) T helper/inducer cell, (Leu 2A) T suppressor/cytotoxic cell, (Leu 7) natural killer cell, (B1) B cell and (T6) Langerhans cell were used to identify the mononuclear cells in the biopsies. We compared the results with our previous study of allergic contact dermatitis. In both types of contact dermatitis, there was a perivascular infiltrate of T-lymphocytes with helper/inducer cells predominating. B cells were absent and natural killer cells were absent or sparse. The numbers and distribution of Langerhans cells were significantly different between allergic and irritant contact dermatitis. In irritant dermatitis the Langerhans cells declined in the epidermis and appeared diffusely in the dermis (days 1, 2). On day 4, the numbers in the dermis decreased abruptly and were again identified in the epidermis. In contrast, in allergic contact dermatitis the Langerhans cells were found predominantely perivascularly in the dermis and did not return to the epidermis until day 14. In summary, Langerhans cells appear to react differently, depending on whether the contactant is an allergen or an irritant.

CONCURRENT SESSION 1B: MELANOMA—MELANIZATION

Congressional

Yoshiaki Hori and Jim Nordlund, Presiding

1. Prostaglandin E$_2$ (PGE$_2$) Induces Proliferation of Murine Epidermal Pigment Cells (EPC). J. J. NORDLUND AND L. A. RHEINS, Dept. of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH.

EPC *in vivo* proliferate in response to injury, inflammation, and ultraviolet light (UVL). The signal which stimulates proliferation has not been identified. Evidence suggests one factor which stimulates proliferation of EPC circulates in the blood (Rosdahl I, JID 73:306, 1979). We have observed that applications of 1–5 μgm arachidonic acid (AA) for 4–7 days to ears of DBA/2 or C$_3$H mice induce a two-fold increase in population density of EPC. Results of tritium-labelled thymidine studies document the AA-induced increase of dopa positive cells in part is due to proliferation of EPC. To determine whether AA-stimulated proliferation directly or via prostaglandins (PG) or leukotrienes (LT), we perform the following experiments. Murine pinnal skin was treated for 14 days with applications of 0.1, 1.0, or 10 μgm of one of the following agents dissolved in 50% DMSO: PGE$_2$, PGF$_{2\alpha}$, PGD, prostacyclin, thromboxane-A analog, LTC, or DMSO only. Only PGE$_2$ significantly increased EPC by day 14. PGE 10 μmg increased EPC from 224/mm^2 to 400/mm^2 ($p < 0.01$). In a repeat study, 50 μgm PGE$_2$ increased EPC from 288/mm^2 to 464/mm^2 ($p < 0.01$) after 14 days. Results of preliminary thymidine-labelling studies indicate PGE stimulated proliferation of EPC. Histologic examination indicated that PGE also stimulated melanogenesis. None of the agents produced cutaneous inflammation. We conclude that PGE is one agent which can stimulate EPC proliferation and melanogenesis. We propose that *in vivo* injury, inflammation, or UVL, all of which stimulate synthesis of PGE in the skin, may cause proliferation of EPC and melanogenesis by this mechanism.

2. Effect of Forskolin on Pheomelanogenesis in Mice. TAKUJI TAKEUCHI AND HIROAKI YAMAMOTO, Dept. of Biological Science, Tohoku University, Sendai, Japan.

Mammalian melanocytes are capable of producing two types of pigments, black or brown melanin (eumelanin) and yellow melanin (pheomelanin). These melanins are different from each other in their chemical and physical properties. In the house mouse, the types of melanin produced in hair-bulb melanocytes is determined by the agouti (*a*) and extension (*e*) loci. Coat color of the mutants at the *a*-locus ranges from extreme black (non-agouti, *a/a*) in which only eumelanin is deposited, to yellow (lethal yellow, A^y/a) which is composed mainly of pheomelanin. On the other hand, the recessive yellow (*e*) gene, which is an allele at the extension locus, is epistatic to the alleles at the *a*-locus. Therefore, mice of genotype *e/e* exhibit hairs pigmented mainly with pheomelanin regardless of their *a*-locus genotype.

Previous studies have shown that the alleles at the *a*-locus exert their effect on melanocytes indirectly by modifying the local tissue environment. On the other hand, the *e*-locus is considered to function within melanocytes. We have examined the effect of disbutyryl cyclic AMP (DbcAMP), α-melanocyte stimulating hormone (α-MSH) and forskolin, a potent activator of membrane-bound adenylate cyclase, on pheomelinin synthesis in lethal yellow and recessive yellow mice. DbcAMP induced eumelanin synthesis in hair-bulb melanocytes of recessive yellow (*e/e*) mice *in vitro*, whereas α-MSH and forskolin did not. In contrast, the melanocytes of letal yellow mice (A^y/a) produced eumelanin in response to all DbcAMP, α-MSH and forskolin. These results seem to indicate that the *e*-locus controls a mechanism involved in the α-MSH receptor-adenylate cyclase system.

3. Identification of 5-S-Cysteinyldopa (5-SCD) by High Performance Liquid Chromatography (HPLC) in Biopsies from Patients with Dysplastic Nevus. M. M. HALLDIN, W. CRUTCHER, T. KAWASHIMA, AND K. FUKUYAMA, Depts. of Dermatology and Pharmaceutical Chemistry, University of California, San Francisco, CA.

Pathological examinations have shown that lesions of dysplastic nevi may be transformed into malignant melanomas. In this study we investigated the chemical evidence of this progression by measuring the content of 5-SCD, a precursor of pheomelanin reported to be the

characteristic product of melanoma cells. Biopsies were taken from involved and uninvolved skin of six patients clinically and pathologically diagnosed as having dysplastic nevi and frozen until analyzed. The tissue samples were homogenized in 0.4 N HCl containing α-methyldopa as the internal standard, and centrifuged at 37,000 rpm for 30 min. The catecholic compounds extracted were adsorbed on a phenylboronate column (pH 8.0) and quantitatively eluted with 0.1 N HCl. The eluent was then analyzed electrochemically by reverse-phase HPLC and the amount of 5-SCD was quantified.

5-SCD was detected in five biopsies from involved skin of two patients. The concentration of 5-SCD varied between 9–40 ng/μg acid soluble protein. No 5-SCD was found in uninvolved skin of the same patients nor in the seven biopsies from involved skin of the four other patients. The skin lesions with and without 5-SCD were clinically and pathologically indistinguishable. The findings indicate that metabolic changes which take place in melanoma cells occur in cells of some dysplastic nevi. The detection of 5-SCD may be considered to be an indication that those dysplastic nevi have a higher risk of malignant melanoma transformation.

4. Glycosylation-dependent Tyrosinase-transfer Steps in Melanogenic Compartments Revealed by Differential Carbohydrate Processing Inhibition Using Deoxynojirimycin and Monensin. GENJI IMOKAWA AND YUTAKA MISHIMA, Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan.

Maturation and translocation of tyrosinases from ribosomes to premelanosomes is key process involving multisteps of their glycosylation. This paper reports our further findings on the differential biological function of various steps in glycoprotein-processing of tyrosinases and premelanosomes occurring in pigment cells. Experiments were carried out using the recovery process from interrupted melanization induced in cultured B-16 melanoma cells by the inhibitor of core carbohydrate synthesis, glucosamine. Treatment of these re-melanizing cells with carbohydrate processing inhibitors, deoxynojirimycin (dNM) and monensin (MS) at 1–2 mM and $3–5 \times 10^{-8}$ M respectively, in the presence of 2mM theophylline almost completely inhibits the re-appearance of the pigment 48–72 hrs after removal of glucosamine. Recovery dynamics of tyrosinase isozymes in large and small granule fractions was analyzed by SDS-PAGE after deoxycholate solubilization. The interruption of pigmentation recovery by the early processing inhibitor (dNM) is characterized by no recovery of T_3 tyrosinase, whereas the late early processing inhibitor (MS) causes an increased recovery of T_3 tyrosinase. These interrupted recoveries are ultrastructurally characterized by a selective loss of melanin deposition within premelanosomes (dNM, MS) and a marked dilation of Golgi cisternae and premelanosomes (MS). Dopa reaction of dNM-treated cells shows predominant localization of dopa melanin in GERL and coated vesicles in contrast to its marked localization in premelanosomes within MS-treated cells. These findings suggest the essential role of the early step of carbohydrate processing for tyrosinase transfer, forming premelanosomal membrane bound T_3 tyrosinase.

5. Monoclonal Antibodies to Mouse T4-Tyrosinase Identify Human Melanocytes. Y. TOMITA, P. M. MONTAGUE, AND V. J. HEARING, Dermatology Branch, National Cancer Institute, NIH, Bethesda, MD.

Tyrosinase is the enzyme essential to pigment formation in melanocytes, which occur primarily in the skin, hair bulbs and eyes. This enzyme has been known to show electrophoretically four forms; T1-, T2-, T3-, and T4-tyrosinase. T4-tyrosinase is a mature form bound to melanosomes and the other forms are thought to be precursors or degradation forms of T4 enzyme. Three hybridomas, TMH-1, TMH-2 and TMH-3, which produce monoclonal antibodies directed against tyrosinase, were obtained by the fusion of SP2/o myeloma cells and lymphocytes of rats hyperimmunized with purified melanosomal T4-tyrosinase. These three monoclonal antibodies bound specifically to the mature, T4 form of tyrosinase, and did not bind to any forms of the enzyme. The enzymological and electrophoretical differences between T1 and solubilized T4 from melanosomes had not been known, but these monoclonal antibodies could clearly distinguish them. Epitope mapping studies have shown that at least two different immunological determinants on tyrosinase are recognized by these antibodies. All three antibodies showed positive immunofluorescence staining of pigmented mouse and human melanocytes from various sources, including *in vivo* and *in vitro* B16 mouse melanoma, human epidermal and nevus melanocytes. The antibodies did not crossreact with unpigmented cells, including K1735 amelanotic melanoma cells, albino mouse skin or eye tissue, fibrosarcoma cells, rat fibroblasts, or epidermal keratinocytes. These monoclonal antibodies are sensitive, highly specific probes for mammalian pigmented melanocytes. Furthermore, these antibodies are expected to be extremely useful in the study of the maturation of tyrosinase in the melanocytes.

6. Reduced DNA Repair in Cultured Melanocytes and Nevus Cells from a Patient with Xeroderma Pigmentosum. K. H. KRAEMER, M. HERLYN, S. YUSPA, W. CLARK, K. TOWNSEND, M. GREENE, G. NEISES, AND V. HEARING, NCI, Bethesda, MD, and Wistar Institute and University of Pennsylvania, Philadelphia, PA.

Patients with the autosomal recessive disease xeroderma pigmentosum (XP) have been reported to have more than a 1000-fold increased risk of cutaneous melanoma in addition to a similar increased risk of basal cell and squamous cell cancer (*Carcinogenesis 5*:511, 1984). We utilized recent advances in cell culture techniques permitting the culturing of human melanocytes and nevus cells to determine whether these melanoma precursor cells in culture express the XP DNA repair defect. A 12 year old girl with XP and 4 nevi greater than 10mm (2 present since birth and 2 appearing later) on her torso. At excision, all were histologically similar with nevus cells extending to the reticular dermis. Melanocytes were cultured in medium supplemented with cholera toxin and TPA (PNAS *79*:2018, 1982). Subsequently, melanocytes were selected by treatment with geneticin (In Vitro *20*:447, 1984). Nevus cells were cultured using a mixture of Dulbecco's MEM and Ham's F10 medium (Cancer Res *43*:5502, 1983). Cultured melanocytes showed typical dendritic morphology, contained mature melanosomes by electron microscopy and reacted with monoclonal antibody to tyrosinase. Nevus cells were spindle shaped, contained few melanosomes, had no tyrosinase activity but expressed nevus cell-associated antigens in mixed hemadsorption assay with monoclonal antibodies. DNA repair was measured by unscheduled DNA synthesis (UDS) following 30J/m^2 UV. Nevus cells, melanocytes, and fibroblasts from the XP patient had 20–50% of normal fibroblast UDS. Following 6J/m^2 UV exposure, proliferative ability of XP nevus cells and fibroblasts was reduced to 10% of that of normal fibroblasts. Thus, cultured XP melanocytes and nevus cells express the characteristic XP DNA repair defect.

7. Low Level of Potentially Lethal Damage Repair (PLDR) of Melanoma Cells after Thermal Neutron Capture Therapy. MASAMITSU ICHIHASHI, HIROSHI UTSUMI*, MASATO UEDA, KAZUHITO HAYASHIBIE, SUSUMU HATTA, AND YUTAKA MISHIMA, Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan, and * Radiation Biology Center, Kyoto University, Kyoto, Japan.

In vivo and in vitro selective killing effects of thermal neutron capture therapy on malignant melanoma have been demonstrated by us using $^{10}B_1$-paraboronophenylalanine ($^{10}B_1$-BPA). In the present study, potentially lethal damage repair of B-10 melanoma cells by thermal neutron capture therapy was investigated to evaluate a high killing effect of the therapy in comparison with conventional radiation therapy. $^{10}B_1$-BPA (0–50 μg/ml) was used for pre-treatment of the cells. Thermal neutron source had a flux of 1.09×10^9n/cm^2/s. Exposure to X ray was done with soft X ray (dose rate of 1,500 rad/min). Killing effect of treatments on melanoma cells was determined by colony formation technique. Fast type PLDR (F-PLDR) was estimated by seeding irradiated cells immediately followed by treatment with medium containing 2 or 3 mM caffeine for 20 h after irradiation. Slow type PLDR (S-PLDR) was studied by delayed plating of the cells which had been incubated with the condition medium after irradiation for 0 to 6 h. Post-irradiation exposure to caffeine reduced the survival of X ray and of thermal neutron irradiated cells. However, the D_o of $^{10}B_1$-BPA plus thermal neutron was only slightly reduced further by the exposure to 3 mM caffeine. Effective S-PLDR was observed after exposure to X ray, but not observed after exposure to either thermal neutron or $^{10}B_1$-BPA plus thermal neutron irradiation. These results of F-PLDR and S-PLDR support radiobiologically a higher killing effect of thermal neutron capture therapy using $^{10}B_1$-BPA on melanoma cells than that of X ray irradiation.

8. Photoprotective Role of Epidermal Melanin Granules Against Ultraviolet Damage and DNA Repair in Guinea Pig Skin. TAKATOSHI ISHIKAWA, KEN-ICHI KODAMA, AND JIRO MATSUMOTO*, Dept. of Experimental Pathology, Cancer Institute, Tokyo, Japan, and * Dept. of Biology, Keio University, Yokohama, Japan.

We previously developed a quantitative autoradiographic technique with special forceps for measuring unscheduled DNA synthesis (UDS)

in mouse skin after treatment with ultraviolet light *in vivo*. By this method, we investigated the relationship between the protective role of melanin and UV-induced DNA repair in black-and-white guinea pigs. Flat areas containing a sharp border between pigmented and unpigmented skin were selected. The skin of the selected areas was shaved and irradiated with short-wave UV (254 nm) or UV-AB (270 to 440 nm, emission peak at 312 nm) at various doses. Immediately after irradiation, the skin was clamped off with forceps, and an isotonic aqueous solution of (methyl-^{3}H)thymidine was injected s.c. into the clamped off portion. UDS was clearly demonstrated as silver grains in this portion of the skin after irradiation with 254 nm UV or UV-AB. Errors due to individual differences were avoided by comparing the intensities of UDS in basal cells from pigmented skin and unpigmented skin of the same animals.

Unexpectedly, in groups of animals treated with 254 nm UV or UV-AB, no difference in UDS in pigmented and unpigmented skin was seen at any UV dose. These results suggested that epidermal melanin granules do not significantly protect DNA of basal cells against 254 nm UV or UV-AB irradiation.

Results of a study on the effect of the wavelength of irradiation on the UDS response of albino guinea pigs are also reported.

9. Expression of Vitiligo-related Pigment Cell Differentiation Antigens on Melanoma Cells After Phenotypic Reversion by Contact Inhibitory Factor. G. LIPKIN, G. K. NAUGHTON, M. ROSENBERG, AND J-C. BYSTRYN, Dept. of Dermatology, New York University School of Medicine, New York, NY.

Most vitiligo sera contain antibodies to surface antigens on pigmented human melanocytes which are not present on human or mouse amelanotic melanoma cells. A density-dependent line of hamster melanocytic cells (FF) releases into its conditioned medium (CM) a contact inhibitory factor (CIF), which reversibly restores density-, anchorage- and serum-dependent growth to hamster, murine and human melanoma cells. Ability of CIF-containing CM to induce expression of another phenotypic characteristic of differentiated pigment cells, viz., vitiligo antigens, on hamster and mouse amelanotic melanoma cells was investigated. Vitiligo and normal sera were reacted with CIF-treated and untreated hamster and mouse amelanotic melanoma cells in an indirect immunofluorescent assay. About 80% of these cells expressed vitiligo antigens in a granular surface staining pattern after, but not before, CIF-induced contact inhibited growth. Less than 5% of control human and mouse amelanotic melanoma cells expressed these antigens. Similar results were obtained with an enzyme-linked immunosorbent assay. By SDS-PAGE and radioautography the CIF-induced antigens were similar to the vitiligo antigens expressed on pigmented melanocytes. Since the vitiligo antigens appeared without pigment induction, we conclude that both are distinct aspects of differentiated function which may be non-coordinately expressed. Expression of pigment cell differentiation antigens on mouse and hamster melanoma cells is an additional component of a pleiotypic response to CIF by melanoma cells, confirms the lack of species specificity of both CIF effects and vitiligo antigens, and provides further evidence for plasticity and potential reversibility of the malignant phenotype.

10. Radioimaging of Malignant Melanoma Xenografts in Nude Mice Using ^{111}In-labelled Monoclonal Antibody (225.28S) Against Human Malignant Melanoma. TETSUZAN KANAMARU, KOICHIRO KAMEYAMA, SHIN-ICHIRO TAKEZAKI, KAZUSHIGE YODA, AND KATSUMI ISHII, Depts. of Dermatology and Radiology, Kitasato University School of Medicine, Sagamihara, Japan.

By using ^{111}In-labelled monoclonal antibody (225.28S) against human malignant melanoma, we examined its availability for radioimmunodetection of human melanoma xenografts in nude mice. 225.28S reacts with most melanoma cell lines, but not carcinoma cell lines or normal human cells (Int. J. Cancer, 28: 293–300, 1981). Two human malignant melanoma cell lines used in this study were KHm-¼ (antigen-positive) and KHm-3/ps (antigen-negative). Monoclonal antibody was conjugated with DTPA first, and then labelled with ^{111}In. After these procedures, labelled 225.28S retained its binding activity against KHm-¼ cells *in vitro*. Nude mice, bearing melanomas at the right hind legs, were i.p. injected with 300 μCi (60 μg) of labelled 225.28S. Tumor images were taken with Gamma camera at 24 hrs intervals. Clear tumor image was obtained even at 24 hrs after injection. Specific localization was confirmed by the absence of imaging in the control mice, bearing antigen-negative melanoma cells (KHm-3/ps). An example of tissue distribution of the labelled antibody, in terms of tumor to tissue ratio at day 3 is as follows: Tumor/intestine 20/1; tumor/liver 10/1; tumor/blood 8/1; tumor/muscle 8/1; tumor/heart 3/1; tumor/kidney 2/1. This study demonstrates the future applicability of monoclonal antibody 225.28S for radioimmunodetection of metastatic lesions in melanoma patients.

11. Characterization of the Induction of Class II HLA Antigens by Recombinant Immune Interferon in Melanocytes. MASAYUKI TSUJISAKI, MUNEO IGARASHI, MAGDA EISINGER*, AND SOLDANO FERRONE, Dept. of Microbiology and Immunology, New York Medical College, Valhalla, NY, and *Dept. of Cell Biology and Genetics, Memorial Sloan Kettering Institute, New York, NY.

Treatment of human melanocytes with recombinant immune interferon (IFN-γ) reduces their expression of a high molecular weight melanoma associated antigen, increases their expression and shedding of Class I HLA antigens and expression and shedding of Class II HLA antigens. Analysis of the induction of Class II HLA antigens by IFN-γ in melanocytes has shown that i) the effect is time and dose dependent, ii) the gene products of the HLA-DR locus are more susceptible to induction by IFN-γ than those of the HLA-DQ locus, as indicated by their appearance on a lower percentage of cells and the higher dose of IFN-γ required to induce their expression than that of HLA-DR antigens, iii) melanocytes from different donors differ in thier susceptibility to modulation by IFN-γ, iv) the 2-dimensional gel electrophoretic profile of HLA-DR antigens synthesized by IFN-γ treated melanocytes is similar to that of B lymphoid cells derived HLA-DR antigens, and v) the effect of IFN-γ is not restricted to Class II HLA antigens, since IFN-γ treated melanocytes acquire a 100K melanoma associated antigen recognized by monoclonal antibodies. Characterization of the induction of Class II HLA-antigens in melanocytes by IFN-γ will contribute to our understanding of the mechanism(s) underlying the unexpected appearance of Class II HLA antigens in melanoma cells.

12. Differences of Lectin (Vicia Villosa Agglutinin) Binding Ability to High-(F10) and Low-(P) Metastatic B16 Mouse Melanoma Cells. HACHIRO YAMANAKA AND YUTAKA MISHIMA. Kanebo Institute for Cancer Research, Osaka, Japan, and Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan.

Modification of cell surface components by neuraminidase and tunicamycin has been shown to reduce blood-borne tumor arrest and subsequent metastasis formation in the lung. To investigate this problem, qualitative and quantitative differences between cell surface glycoconjugates of high- and low-metastatic sublines of B16 cells, were analyzed using fluorescein-labeled (FITC-) lectins. High-metastatic B16-F10 established by Fidler produces over 200 nodules on the lung surface 2 weeks after intravenous inoculation of 10^5 cells, while low-metastatic B16-parent line (P) produces less than 1 nodule. Of lectins we tested (PNA, SBA, Con A, WGA etc), using fluorescence microscopy, Vicia villosa agglutinin (VVA) was found to bind to high- and low-metastatic sublines differently. P binds VVA substantially while F10 does not. Cells stained with FITC-VVA were further analyzed by fluorescence activated cell sorter (FACS). The frequency of VVA-positive P cells was 65% while that of F10 was 5%. After removal of sialic acid with neuraminidase the VVA-positive P increased to 79% while that of F10 reached 55%. We found that high- and low-metastatic sublines of B16 can be distinguished by VVA binding ability. It is concluded that low-metastatic cells have both sialylated and nonsialylated glycoconjugates which have specific recognition sites for VVA while high-metastatic cells have only sialylated ones. Sialylation of glycoconjugates involving VVA receptor sites may possibly associated with metastatic potential. On the other hand, although peanut lectin (PNA) binding ability has been found to be variable among subclones of F10 cells, a direct correlation with metastatic ability was not found.

13. Growth and Metastasis of B16 Melanomas in C57BL/6J Normal and vi/vi Mutant Mice. AARON B. LERNER, TETSUO SHIOHARA, RAYMOND E. BOISSY, AND KIRSTEN A. JACOBSON, Dept. of Dermatology, Yale University School of Medicine, New Haven, CT.

It has been suggested on the basis of clinical experience that melanocytes of people with vitiligo, or a genetic background for vitiligo, are predisposed to becoming transformed to melanomas (Lerner et al, 1984). As a first approach in testing this hypothesis, growth rates and the incidence of metastases of injected B16 melanomas were determined in normal C57BL/6J (B6) mice and mice of the "vi/vi" mutant line. Vi/vi mice show progressive idiopathic pigment loss. The mutation occurred spontaneously in B6 mice and may have provided us with a much needed murine for vitiligo. To compare tumor growth in B6 and

vi/vi mice (n = 9 each), cultured B16 melanoma cells (1–5 × 10^6 cells/mouse ear) were injected into the dorsal surface of both pinnae, and tumor growth was assessed by measuring tumor diameter. The recipients were between 12 and 16w of age, during active depigmentation of the vi/vi mice. B16 melanoma cells grew significantly faster in the mutant mice than in age- and sex-matched B6 controls and vi/vi mice that received a total of 5 × 10^6 melanoma cells died earlier than relevant controls (12–16d vs 20–23d post injection). When similar experiments were performed with young mice before the onset of depigmentation, the differences between vi/vi and control mice were less pronounced. Experimental metastases of bloodborne melanoma cells were determined by a lung colony assay. 4 × 10^6 melanoma cells were injected into the tail veins of 16w old vi/vi and B6 mice (n = 6 each), and on day 14 pulmonary surface nodules were counted. The number of nodules in vi/vi mice was more than double that in B6 mice (748 ± 60 vs. 284 ± 104). These early results indicate that during the period of progressive depigmentation vi/vi mice are impaired in their ability to suppress the growth of exogenously administered melanoma cells.

14. Time-dependent Suppression of Experimental Melanoma Metastasis and Natural Killer Cell Activation by Interferon. MASATO UEDA, YUTAKA MISHIMA, MASAMITSU ICHIHASHI, AND MANOJ MOJAMDAR, Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan.

The effect of murine α/β interferon (IFN) on experimental metastasis was investigated using B16-F10 melanoma which was established by Fidler as a high lung metastatic subline. Since the outcome of metastasis of blood-borne tumor cells is known to be primarily determined within the first 24 hrs after i.v. inoculation, IFN administration was focused around this critical early phase. To study the in vivo anti-metastatic effect, 2 × 10^4 IU of IFN was once given i.p. either before, during or after an i.v. inoculation of 2 × 10^5 F10 cells into mice. The mice were sacrificed two weeks later, and the number of metastatic colonies on the lung surface were counted. The in vitro effect of IFN on the metastatic capability of F10 cells was assayed by culturing them in an IFN containing medium for 24 hrs. The possible role of NK cells in the anti-metastatic effect of IFN was analyzed by administering anti-asialo GM1 before tumor cell inoculation, and studying NK cell-mediated cytotoxicity in vitro. The inhibition of the lung colony formation by IFN is found to be maximal (80 ~ 98% inhibition) when given 3 hrs prior to tumor cell inoculation. IFN given 12 and 24 hrs prior to as well as simultaneously with tumor cell inoculation, also reduces metastases, but to a lesser extent. When given 2 hrs after the inoculation, no effect is shown. The metastatic potential of the tumor cells by in vitro incubation with IFN is not decreased. The salutary effect of IFN is abolished by anti-asialo GM1 administration. NK cell activity of spleen cells is enhanced after the IFN administration. These data indicate that a prepared host status against the blood-borne tumor cells is established by the IFN administration prior to tumor cell inoculation, and this effect is substantially dependent upon NK cell activity.

15. A Model of Human Melanoma in Cyclosporine Immunesuppressed Rats. MATTHEW M. GOODMAN, CATHERINE A. BIREN, JERRY L. MCCULLOUGH, AND RONALD J. BARR, Dept. of Dermatology, University of California at Irvine, Irvine, CA.

Cyclosporine (CSA) is an effective bone marrow-sparing immunosuppressant. Using CSA we have developed an in vivo rat model of human melanoma. Thirty-six 8mm^3 sections from a human metastatic melanoma xenograft in athymic nude mice were implanted s.c. in 2–4 sites in each of 10 Lewis rats. Treatment with either CSA (25 mg/kg) or vehicle alone resulted in rapidly-growing nodules in 100% of rats in the CSA group after 15–22 days, compared with no tumor growth in controls. Tumors retained pre-transplant gross and microscopic morphology, pigmentation, growth curve and labeling index. After tumor establishment at a CSA dose of 25 mg/kg, a lowered dose of 15 mg/kg or discontinuation of CSA led to complete regression of tumor within 28 days, indicating dose-dependence of the model. A smaller inoculum of 2 × 10^6 cells in suspension was injected s.c. in 25 animals, 15 receiving 15 mg/kg, 4 receiving 25–50 mg/kg, and 6 vehicle controls. No tumor developed in treatment or control groups, indicating importance of inoculum size. However, when simultaneous xenografting of human skin and tumor suspension injection was done, tumors developed in 4/4 sites of treated animals (15 mg/kg) compared with none in vehicle controls or sham incision controls receiving the same dose of CSA. By an unclear mechanism, the presence of xenografted human skin assisted CSA in preventing rejection of the tumor xenograft.

This model offers several advantages over the athymic nude mouse including greater longevity, simpler maintenance (sterile facilities not required), larger surface area, and greater blood volume for serial sampling. This model may prove useful in further characterizing the biology and therapy of melanoma and other cutaneous diseases.

CONCURRENT SESSION 1C: MATRIX PROTEINS, CONNECTIVE TISSUE

Federal

Shingo Tajima and Howard Welgus, Presiding

1. Regulation of Collagen Gene Expression in Fibrotic Skin Diseases. JOUNI UITTO, R. PATRICK ABERGEL, EUGENE A. BAUER, MON-LI CHU, AND FRANCESCO RAMIREZ, Harbor-UCLA Medical Center, Torrance, CA, Washington University School of Medicine, St. Louis, MO, and UMDNJ-Rutgers Medical School, Piscataway, NJ.

Dermal fibrosis, characterized by collagen accumulation, is the hallmark of several cutaneous diseases. To examine the mechanisms of collagen deposition in the fibrotic skin diseases, fibroblast cultures were established from the skin of patients with progressive systemic sclerosis, morphea, scleredema, familial cutaneous collagenoma, connective tissue nevi of the collagen type or keloids. Collagen production was assayed by synthesis of [^{3}H]hydroxyproline, and type I and III procollagen mRNA levels were determined by dot blot hybridizations using human proα1(I), proα2(I) and proα1(III) collagen specific cDNA probes. The collagen production in fibroblast cultures from the fibrotic diseases was increased up to 6-fold over the controls, and a relatively good correlation between the collagen production and type I collagen mRNA levels was noted. The type I/III procollagen mRNA ratio in control fibroblast cultures was 5.9 ± 1.6 (mean ± S.D.). The corresponding ratio in keloid cell cultures was markedly increased, while slightly decreased values were noted in case of morphea and familial cutaneous collagenoma; the values in other cultures were within the normal range. The results suggest that procollagen production in fibroblast cultures derived from fibrotic skin diseases reflects elevated levels of the corresponding procollagen mRNA. The increased mRNA abundance may result from enhanced transcriptional activity of the corresponding gene or alternatively reflects increased stability of the mRNA molecule.

2. A 1-Year Study of Collagen Synthesis, Collagen Content, and Microscopic Fibrosis in Irradiated Mouse Skin. RENATO G. PANIZZON, WAYNE R. HANSON*, FREDERICK D. MALKINSON, AND DAVID E. SCHWARTZ†, Depts. of Dermatology, * Therapeutic Radiology, and † Biochemistry, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

Late effects in normal tissues contribute the main complications of radiation therapy. The most common is fibrosis. Earlier contradictory reports prompted us to study the patterns of post-radiation fibrosis in skin. Mice were irradiated to the thorax with a dual head ^{137}Cs source, receiving 0, 5, 10, 15, or 25 Gy (2500 r). Skin specimens were taken at 1–48 weeks later. For collagen synthesis minced skin specimens were incubated in medium containing ^{3}H-proline and assayed for radioactive hydroxyproline. In 5μ tissue sections the ratio of collagen to total protein was determined. Sections were also "blindly" evaluated microscopically for fibrosis. Fibroblast subcultures from skin explants were incubated in medium with ^{3}H-proline and assayed for ^{3}H-hydroxyproline. A dose dependent increase in collagen synthesis was seen at 1, 4, and 12 weeks. Such increases ranged to 50% higher than controls. Similar time and dose dependent patterns of increased collagen content were found in tissue sections. In contrast to the biochemical detection of increased collagen synthesis and content within 1 week at all dose levels, microscopic fibrosis was first detected 4 weeks postradiation and then just in the 25 Gy animals. At all other dose levels such changes were not found until 3 months or more post-radiation. In cultured fibroblast collagen synthesis increased three-fold by 12 weeks post-exposure. We conclude that: 1) cutaneous collagen synthesis and content increased within one week post-radiation and remained elevated, even at 5 Gy for almost a year; 2) microscopic detection of fibrosis is an inadequate end point; 3) subcultured irradiated fibroblasts continued to synthesize excessive collagen; and 4) postradiation fibrosis is an early, not a late event.

3. Stimulation of Collagen Production in Human Skin Fibroblasts by Ascorbic Acid is Unrelated to Intracellular Degra-

dation. J. C. GEESIN, S. MURAD, AND S. R. PINNELL, Div. of Dermatology, Duke University, Durham, NC.

We have previously reported a specific stimulation of collagen production in human skin fibroblasts by ascorbic acid (PNAS 78, 2879, 1981). Cells in culture degrade from 10 to 40% of newly synthesized collagen before secretion. Although the mechanism is unknown, this process may control the quality of collagen produced. Since ascorbic acid is a cofactor in the synthesis of hydroxyproline which is known to stabilize the collagen triple helix, the underhydroxylated collagen produced in ascorbate deficiency may be less stable and thus more susceptible to intracellular degradation. To test this hypothesis, we have measured intracellular procollagen degradation in ascorbate-stimulated cells. Confluent cells were labeled for 18 hr with repurified [2,3-^{3}H] proline. Intracellular degradation was determined by comparing hydroxyproline in low molecular weight peptides to total hydroxyproline. Cells treated with 100 μM ascorbic acid for 72 hr degraded 11.2 ± 0.8% of newly synthesized collagen and the untreated cells degraded 11.2 ± 0.9%. In the absence of ascorbic acid, the collagen synthesized by cultured fibroblasts is hydroxyproline deficient but not hydroxylysine deficient. When cells were labeled with [^{14}C]lysine and intracellular degradation determined by measuring hydroxylysine, there was again no significant difference in degradation between ascorbate-treated and untreated cells (23.5 ± 4.7% vs 24.9 ± 6.9%). The difference in values obtained by the two methods remains unexplained. Nevertheless, the results suggest that the increase in collagen production noted in cells grown in the presence of ascorbic acid is due to an induction of collagen synthesis rather than a reduction in intracellular degradation.

4. Phorbol Myristate Acetate Induces Increased Production of Collagenase Inhibitor in Human Skin Fibroblasts. S. D. CLARK, S. M. WILHELM, G. P. STRICKLIN*, AND H. G. WELGUS, Div. of Dermatology, Washington University School of Medicine and The Jewish Hospital of St. Louis, MO, and *Div. of Dermatology, University of Tennessee, Memphis, TN.

Phorbol myristate acetate (TPA), a tumor promotor known to stimulate collagenase production in fibroblasts, synovial cells, and endothelial cells was studied with regard to its ability to regulate the expression of a collagenase inhibitor secreted by human skin fibroblasts (HSFs). HSFs were grown to confluence and treated with concentrations of TPA ranging from 10^{-11} to 10^{-7} M in serum-containing and serum-free medium. Conditioned medium was analyzed by ELISA for both immunoreactive collagenase and collagenase inhibitor. Concentrations of TPA that produced a 2- to 7-fold increase in collagenase stimulated production of inhibitor by a factor of ~2 in serum-containing medium and ~4 in serum-free medium. Production of inhibitor was 50% of maximal at 5×10^{-8}M and maximal at 10^{-7}M TPA. This dose-dependent effect was very similar to that observed for collagenase production. Total protein synthesis by the TPA-conditioned cells, as studied by incorporation of ^{3}H-leucine into newly synthesized protein, was not significantly increased, nor was cellular DNA content. The onset of phorbol's effect on inhibitor production occurred at 8 hr, was maximal by 24 hr, and inhibitor levels in culture medium reached a plateau after 48 hr. In control cells, maximal inhibitor concentrations were not attained until 72 hr. Exposure of cells to 35 S-cysteine followed by immunoprecipitation using inhibitor-specific antibody demonstrated that phorbol's effect was mediated via an increased synthesis of new inhibitor protein. We conclude that in response to the tumor promotor TPA, collagenase and collagenase inhibitor expression are regulated in a coordinate manner.

5. Monoclonal Antibodies Demonstrating Cross-reactivity Between Group A Streptococci and Human Skin. R. SWERLICK, M. CUNNINGHAM, P. SEXTON, L. DEBAULT, AND N. HALL, Depts. of Dermatology, Microbiology and Immunology, and Pathology. University of Oklahoma Health Sciences Center and Veterans Administration Medical Center, Oklahoma City, OK.

Cross-reactivity between human skin and group A streptococci has been implicated in the pathogenesis of several cutaneous disorders. For this reason, three monoclonal antibodies, previously characterized and found to bind to streptococcal membranes, were reacted with sections of normal human skin and with acetone fixed cultured human cells which represented the major cell populations of human skin (keratinocyte, fibroblast and endothelial). These monoclonal antibodies, 5428, 4982, and 3622, were produced by hybridomas from fusions of X63-AG8654 murine myeloma cells and spleen cells from BALB/c mice immunized with group A streptococcal membranes. The cell lines examined were A-431 squamous carcinoma cells, human skin fibroblasts, and two thrombomodulin producing cell lines. Immunofluorescence microscopy demonstrated distinct patterns of staining for each monoclonal in both skin sections and fixed cells. Monoclonal antibody 5428, previously shown to react with cardiac and skeletal muscle myosin as well as streptococcal membrane components, exhibited nuclear binding in all specimens examined. It also appeared to bind to cytoskeletal elements in fixed cultured cells, except for the A-431 line. Western immunoblots of SDS solublized cell extracts from endothelial and fibroblast lines showed two major bands at 45K and 100K daltons when reacted with monoclonal 5428, while a single band was noted at 100K daltons when this antibody was reached with immunoblots of A-431 cells. Human skin appears to have antigens which cross react with determinants of group A streptococci.

6. Cell Renewal in Human Epidermis. MARCELLE RÉGNIER, PIERRE VAIGOT, MICHEL DARMON, AND MICHEL PRUNIÉRAS, Dept. of Cell Biology, Centre International de Recherches Dermatologiques (C.I.R.D.), Valbonne, France.

To understand the process of cell renewal in human epidermis, one needs an accurate evaluation of the growth potential of basal versus suprabasal cell population. In the present study, human epidermal cell suspensions were stained with propidium iodide. The cell cycle was analyzed with a flow cytometer and the cells belonging to the G_0/G_1, S and G_2/M fractions were sorted out directly on glass slides. An anti-keratin monoclonal antibody ($K1_1$) reacting only with suprabasal epidermal layers and an antibody reacting with bullous pemphigoid antigen (BPA), a classical marker of basal cells (BC), were used to double label the sorted cells. The results show that the cycling cell pool of human epidermis is composed of two major subpopulations, one Kl_1-BPA+ accounting for 45.8 ± 6.7% and one and Kl_1 + BPA− accounting for 50.2 ± 7%. The number of cells in G_0/G_1, S and G_2/M is practically the same in the two subpopulations (cycling pool* 3 to 4%). A third population of cells is both Kl_1 + BPA+. This small fraction accounts for 4.2 ± 1.6%. Surprisingly, this Kl_1 + BPA + population presents an important cycling pool (20%).

In conclusion, i) basal cells (BPA+) are heterogeneous, ii) there exists a small population with a high cycling fraction, iii) this subpopulation contains keratins recognized by Kl_1 monoclonal antibody.

* Cycling pool: number of cells in S + G_2/M phases.

7. A Study of Ultrathin Frozen Sections of Epidermal Keratinocytes in Human Skin. HIROYUKI SUZUKI AND SHIGEO KAKIMI, Depts. of Dermatology and Anatomy, Nihon University School of Medicine, Tokyo, Japan.

Cryomicrotomy has for some time been considered to offer new possibilities for the elucidation of cellular fine structure. The present study set out to investigate the suitability of using cryomicrotomy and negative staining techniques for electron microscopic observation of epidermal keratinocytes in human skin. Specimens taken from human skin were fixed in 2.5% glutaraldehyde and frozen after immersion in sucrose. Ultrathin frozen sections cut with a cryomicrotome were negatively stained with 2% phosphotangstic acid, 1% uranyl acetate and 2% ammonium molybdate, and examined with an electron microscope. The most distinctive features were the fine structures of the desmosomes. Fine filaments 7–8 nm in diameter perpendicularly linked the tonofilament bundle to the attachment plaque which was composed of an aggregation of granular and filamentous structures. Similar fine filaments also linked the attachment plaque to the cell membrane. In the lamellar granules (MCG), each lamellated component was observed more clearly than in conventional electron microscopy. Keratin filaments in the cornified cells often appeared as tubular pipe-like structures in cross section. These results indicate that cryomicrotomy of ultrathin frozen sections can produce images of the fine structure of cellular components such as desmosomes in human epidermal keratinocytes that are not attainable using conventional techniques.

8. A New Monoclonal Antibody to Purify and Culture Basal Keratinocytes. A. B. GOTTLIEB, D. N. POSNETT, T. HORIKOSHI, M. K. CROW, L. MAYER, AND D. M. CARTER, Laboratories of Investigative Dermatology and Immunology, The Rockefeller University, New York, NY.

We have made a new monoclonal antibody (Mab) EL-2, and used it with a simple rosetting procedure to purify and culture basal keratinocytes. Immunoflourescence of cell suspensions and immunoperoxidase staining of tissue sections demonstrate that EL-2 reacts with malignant cell lines, activated lymphocytes and monocytes, and basal

keratinocytes. Sequential immunoprecipitation studies demonstrate that Mab EL-2 and Mab 4F2 detect the same membrane protein. However, we have extended previous studies by making the new observation that both EL-2 and 4F2 react with cultured melanocytes and endothelial cells.

Basal keratinocytes were purified from single-cell epidermal suspensions by incubation with Mab EL-2 followed by rosetting with rabbit antimouse IgG antibodies covalently linked to bovine red blood cells. Rosetting (basal) keratinocytes were separated from EL-2 negative cells by Ficoll gradient cenrifugation. We obtained basal keratinocyte populations of >90% purity as assessed by reactivity with EL-2 and another basal keratinocyte-specific Mab, HC1. Langerhans cell, fibroblast and melanocyte contamination was negligible. Cultures of basal keratinocytes incorporated more ^{3}[H]-thymidine and contained more cells than cultures of unseparated keratinocytes.

Cultures of purified basal keratinocytes are being used as grafts in patients with nonhealing wounds and will be important in biochemical and functional studies of normal skin. EL-2 is being used to characterize disorders of keratinocyte proliferation such as psoriasis.

9. Identification of Human Fetal Periderm with a Monoclonal Antibody. A. T. LANE, M. NEGI, AND L. A. GOLDSMITH, Dermatology Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Human fetal periderm forms a transient covering over the developing epidermis from the 1st to 6th month of human fetal development. We have developed an IgM mouse monoclonal antibody, Peri 1, which specifically stains human fetal periderm by indirect immunofluorescence. Characterization of the complimentary antigen identifies a 36kd protein on immunoblot. The antigen is soluble in SDS and mercaptoethanol. This antigen is absent from human fetal skin after 6 months gestation, human foreskin and adult skin by indirect immunofluorescence. Human foreskin organ explant keratinocytes within 24 hours in culture with definite expression on actively growing keratinocytes at 48 hours and later. Although human fetal skin at term does not express this antigen, human amnion expresses the antigen during fetal development and at term. Immunofluorescent studies of monolayers of human keratinocytes identifies the antigen to be intracellular, concentrated around the periphery of the cells in a pattern different from cytokeratins, vimentin or actin.

Peri 1 represents a monoclonal antibody for an epidermal antigen that is expressed in very early fetal development, not synthesized in neonatal or adult tissue but is re-expressed in epidermal cell culture. Further evaluation of this antigen will increase our information of periderm function and keratinocyte changes in epidermal cell culture.

10. Growth Factors in Blood Platelets that Support and Stimulate Epidermal Cell Outgrowth in Skin Explants. P. A. HEBDA AND W. H. EAGLSTEIN, Dept. of Dermatology, University of Pittsburgh, Pittsburgh, PA.

Platelets contain growth factors (GFs) for dermal components including fibroblasts and smooth muscle cells, and it is thought that these factors may be important wound healing signals. We previously demonstrated that platelets also contain GFs for epidermal cells. We now report the partial fractionation of these epidermal factors. For this work we used explants, prepared from 0.3 mm thick electrokeratome-excised pig skin, to study the effect of platelet fractions on epidermal cell outgrowth, which serves as an in vitro model for epidermal wound healing. Explants were cultured with 0, 1 or 5% fetal bovine serum (FBS) and platelet fractions were prepared by 1) heating at 100°C for 2 min or 5 min and 2) ultrafiltration that nominally excludes above 10 kdaltons or 30 kdaltons. Control cultures were grown with FBS alone. The platelet homogenate contained a component that enabled explants to grow without FBS, to the same extent as FBS controls. This "viability GF" was stable at neutral pH at 100°C for 2 min but not 5 min, was partitioned into the >30 kdalton fraction, and by these criterion is similar to Epibolin and Serum Spreading Factor. The platelet homogenate also contained a component that enhanced the rate of epidermal outgrowth in the presence of FBS or the "viability GF." This "stimulatory GF" was inactivated at neutral pH, 100°C for 5 min, was contained in the 10–30 kdalton fraction, and by these properties is similar to Transforming GF-β and Platelet-derived Epidermal GF Variant. These studies show that platelets contain GFs that may be involved in the regulation of epidermal, as well as dermal, wound healing events. Such factors have potential applications for wound management.

11. Unusual Cell Function of Dermal Fibroblasts Derived from Werner's Syndrome. ATSUSHI HATAMOCHI, KOJI TAKEDA, AND HIROAKI UEKI, Dept. of Dermatology, Kawasaki Medical School, Okayama, Japan.

Werner's syndrome, a premature aging disorder, is much different in clinical characteristics from normal aging. To ascertain these differences we compared the cell function of fibroblasts derived from Werner's syndrome to the cell function of fibroblasts from normal aging. Collagen synthesis, glycosaminoglycans synthesis and fibronectin (FN) were determined on dermal fibroblasts from both Werner's syndrome and the normal control sample. These samples matched according to age, sex and biopsy site. The total synthesized protein was determined by the incorporation of ^{3}H-proline, and the synthesized collagenous protein was measured as labeled hydroxyproline content in the cell layer and medium. Glycosaminoglycans synthesis was measured by the incorporation of ^{3}H-glucosamine. FN released to the medium was measured using enzyme-linked immunosorbent assay. In the 9th population doubling level (PDL), the increased synthesis of total and collagenous protein of fibroblasts from Werner's syndrome was about three times as much as that of the control sample. In the 13th PDL, a similar result was also obtained. The incorporation of ^{3}H-glucosamine of fibroblasts from Werner's syndrome, measured in the 15th PDL, was significantly higher than normal. Several changes in the cell function of fibroblasts with normal aging is already known: a decrease of collagen synthesis, an increase of released FN to the medium and so on. In our study, the collagen synthesis of fibroblasts from Werner's syndrome was higher than that of control sample and the released FN to the medium did not increase in fibroblasts from Werner's syndrome. These results revealed another factor functioning in fibroblasts from Werner's syndrome. This factor causes the changes of cell function.

12. Biochemical Study on a Protein Unique to Cultured Cells from Angiofibroma of Tuberous Sclerosis. TAKASHI UMEDA, TOHRU HARA, TADAO NIIJIMA, YOSHIAKI KOMIYA*, TOMOKO TASHIRO*, AND YASUMASA ISHIBASHI†, Dept. of Urology, *Institute of Brain Research, and †Dept. of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Disoriented behavior of chromosomes has been described by Ishibashi at division of cultured cells from angiofibroma of tuberous sclerosis. Chromosomes in the cells did not collect into a metaphase plate, and after extreme lagging, they either divided into two or more daughter cells mostly in different sizes, or failed to divide, forming multinucleated giant cells. Therefore, a functional defect seems to be present in centromere-microtubule-centriole in the cells. To investigate biochemical defect underlying these histochemical abnormalities, we have studied metabolism of cytoskeletal compositions of these cells. The cultured cells from angiofibromas were labeled with L-(^{35}S)methionine. After being washed, these cells were chased for 3 hrs. Compositions of the labelled protein were analysed by two-dimensional gel electrophoresis with visualization by fluorography. Cultured fibroblast cells from normal skin and fibromatosis were chosen as controls. A protein unique to tuberous sclerosis (Mr = 55,000, pI = 5.6) was found. This protein spot shifted to acidic side after 3 hrs chase. These findings suggest the presence of complex disorders of biochemical metabolism linking the functional defect in centromere-microtubule-centriole system in these cells.

13. Cloning of Chick Elastin cDNA. SHINGO TAJIMA, ICHIRO TOKIMITSU, TAKEJI NISHIKAWA, AND TOSHIO FUKASAWA*, Dept. of Dermatology and *Laboratory of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

Elastin is a major component of connective tissue in aorta, lung and cartilage, and plays an important role to confer elasticity to these organs in the physiological state. In dermal connective tissue, elastin has been identified only by the histological method such as Elastica van Gieson staining and its presence has not been proven in terms of biochemistry. In order to study the metabolism of dermal elastin, we attempted to synthesize and clone elastin cDNA using RNA tropoelastin, the precursor form of elastin, was shown to be actively synthesized in an organ culture of chick embryo, indicating predominant existence of elastin mRNA in that organ. Total RNA was then extracted from aorta from 10 day chick embryo. Elastin mRNA was concentrated by the oligo (dT) cellulose chromatography followed by the sucrose density gradient centrifugation. The amount of elastin mRNA was determined by the cell-free translation system of rabbit reticulocyte lysate. A cDNA library was successfully constructed with the partially purified elastin mRNA by the procedure of Okayama-Berg. An elastin cDNA molecule

with a size of approximately 1.5 Kb was obtained, which hydridized to 24 S RNA in total RNA from chick aorta. The molecule had the unique site for EcoRI, PvuII or PstI.

14. Monoclonal Antibody Recognizing Elastic Fiber Microfibril. N. KAMBE, T. FUKAYA, M. MATSUMOTO, M. ASO, K. ITO, AND K. HASHIMOTO, Dept. of Dermatology, Wayne State University, Detroit, MI and VAMC, Allen Park, MI.

Monoclonal antibody against elastic fiber microfibril has not been reported. We produced a unique monoclonal antibody, NKH-1, which reacted with elastic fiber microfibrils and dermal-epidermal junction (DEJ). Junctional interface substances were extracted from 1 M NaCl-separated dermis with 8 M urea and 0.3 M beta-mercaptoethanol and used as an antigen. The spleen cells from the immunized mouse were fused with mouse SP2/0 myeloma cells employing hybridoma technique. With the immunoperoxidase technique on frozen sections, NKH-1 stained the fine fibers oriented perpendicularly to DEJ in the most superficial layer of the dermis and it also stained DEJ in granular pattern. Immunoelectron microscopic study revealed that the reaction product for NKH-1 was localized on the microfibrils of papillary oxytalan fibers and peripheral microfibrillar mantle surrounding the mature elastic fibers. Interestingly, subbasal lamina area was also stained. The polyclonal antibody to serum amyloid-P component (SAP), which is known to react with elastic fiber microfibril, stained only thicker fibers in papillary and reticular dermis, and DEJ was not stained. From these observations, we conclude that NKH-1 recognizes elastic fiber microfibils and is different from anti-SAP antibody. It is suggested that elastic fiber microfibril-associated antigen also exists at DEJ along the basal lamina.

In application to abnormal conditions, it was found that elastic fibers in pseudoxanthoma elasticum were poorly identified with NKH-1. Interestingly, a proliferation of thin elastic fiber could be demonstrated surrounding individual nests of intradermal nevus.

15. Disoriented Behavior of Chromosomes in Cell Division in Tuberous Sclerosis. YASUMASA ISHIBASHI, KAZUHIKO TAKEHARA, AND ATSUSHI KUKITA, Dept. of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Tuberous sclerosis is a specific hereditary disease characterized by appearance of angiofibromas (AFs) on the face, epileptic attacks and mental retardation. Attention has long been paid to the atypical cells histologically observed in the stroma of AF as they may play an important role in forming the lesions. For the purpose of clarifying the nature of these cells we have investigated DNA contents of the cells cultured from AFs using a flow cytofluorometer, and found that DNA histograms showed distinct abnormalities indicating unequal distribution of DNA in cells. The possibility of abnormal DNA content in cells was further reconfirmed by electron microscopic and immunofluorescent investigations. Namely, in cell division, chromosomes in these cells did not seem to collect into a metaphase plate, and after extreme lagging, they finally either divided into two or more daughter cells mostly in different sizes, or failed to divide, forming multinucleated giant cells. Immunofluorescent techniques using anti-DNA antibody clearly demonstrated the disturbed divisions of chromosomes. Electron microscopic observations of mitotic figures clarified disoriented arrangements of chromosomes associated with the presence of abundant microtubules, most of which seemed to be unrelated to kinetochores of chromosomes. From these findings we concluded that a functional defect is present in "centromere-microtubule-centriole system" in the cells.

CONCURRENT SESSION 1D: PHARMACOLOGY

South American

Kenzo Sato and Hashiro Tagami, Presiding

1. Quantitative Testosterone Stimulation of the Hamster Flank Organ Using Silastic Capsules: Response of Androgen-dependent hair. A. W. LUCKY, J. MCGUIRE, E. NYDORF, G. HALPERT, AND B. NUCK, Depts. of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH, and Yale University School of Medicine, New Haven, CT.

We have studied the response of hair in the female Golden Syrian hamster flank organ by implanting 0.25 to 2.0 cm silastic capsules filled with crystalline testosterone (T) subcutaneously. Hair diameters were measured on paraffin sections cut from the center of the flank organ. Mean hair diameter increased maximally from 24 ± 14 μm to 45 ± 27 μm ($p < 0.001$) using the smallest T capsule for six weeks. Maximal growth of pigment and sebaceous gland was achieved with a 1 cm capsule. Mean hair diameters adjacent to, but outside the sebaceous gland were not significantly affected by T. T caused a shift of hair diameter distribution: small (<40 μm) hairs decreased from 85% to 60%, whereas medium and large (>80 μm) hairs increased from 15% to 25% and from 0% to 15% respectively. The density of hairs under the sebaceous gland was unchanged during three weeks of T stimulation with a 1 cm capsule, although mean hair diameter and sebaceous gland size increased. Topical progesterone, 2.5 mg daily, partially inhibited hair diameter increase.

We conclude that 1) silastic capsules filled with T can provide a quantitative and continuous release of androgen; 2) hair diameter is more sensitive than sebaceous gland or pigment to T; 3) hair diameter increase is specific for hairs directly under the sebaceous gland; and 4) T does not alter hair density. These new methods for delivering androgen and assessing hair response should be useful in studying of the effect of androgens and anti-androgens on hair.

2. Sebaceous Gland Hyperplasia on Rabbit Pinna Induced by Topically Applied Tetradecane. MASAAKI ITO, KATSUHIRO MOTOYOSHI, MEGUMI SUZUKI, FUMIKO SAKAMOTO, THOMOHIRO MARUYAMA, AND YOSHIO SATO, Dept. of Dermatology, Niigata University School of Medicine, Niigata, Japan.

To investigate the histological and ultrastructural changes of the sebaceous glands in the experimental comedogenesis on rabbit pinna model was the purpose of this study. Purified tetradecane was inuncted on the ventral aspect of rabbit pinnas once a day for a week. Histologically, a marked hyperplasia of sebaceous glands, epidermis and follicular epithelium was seen. These remarkably enlarged sebaceous glands were examined with N-(7-dimethylamino-4-methyl-3-coumarinyl)-maleimide (DACM) staining method and transmission electron microscope (TEM). By DACM staining, normal distribution patterns of SH and SS were seen in the peripheral to differentiating layers in the hyperplastic sebaceous glands. In the terminally differentiated layer, the brilliant SH-fluorescence was gradually decreased and the SS-fluorescence was gradually increased in intensity, indicating that the most SH groups in the sebaceous cells were converted to SS linkages before holocrine secretion. By TEM, several cell layers of undifferentiated sebaceous cells were observed at the periphery of the large sebaceous gland. The differentiating sebaceous cells produced a large number of lipid droplets, which were produced in either rough or smooth endoplasmic reticulum. These cells were abruptly converted into homogeneously electron dense cells which formed several layers. These homogeneous cells gradually lost the electron density before holocrine secretion. This is the first report of the sebaceous hyperplasia induced by a topically applied substance on skin surface, except for androgens. The hyperplastic sebaceous glands could serve as a model for investigations of sebaceous cell differentiation and lipid formation.

3. A Potent Topical Steroid with Less Systemic Side Effect, Hydrocortisone 17-Butyrate 21-Propionate (HBP). SUSUMU OTOMO, MAKOTO MURAMATSU, SHOHEI HIGUCHI, YASUO OZAWA, AND IKUO KOYAMA, Research Center, Taisho Pharmaceutical Co. Ltd., Ohmiya, Saitama, Japan.

HBP, which we have recently developed, has a potent anti-inflammatory effect without significant systemic influences by topical administration because upon transferring to circulation it is easily metabolized to de-esterified steroid. To clarify the mechanism of its potent local effect, we have studied affinity to steroid receptor, cell adsorption, metabolism and distributing of HBP. The binding affinity of HBP to steroid receptor was much stronger than that of hydrocortisone and almost equal to that of hydrocortisone 17-butyrate(HB). In the cell absorption study, HBP was adsorbed to polymorphonuclear cells more strongly than HB and betamethasone 17-valerate(BV) in vitro using rat peritoneal exudate cells. HBP was also distributed mainly in the horny layer in the rat and dog skins. HBP was hardly metabolized in horny layer but in viable epidermal layers, it was de-esterified to 17-monoester steroid though about 15% and 50% of steroid in the viable epidermis existed as unesterified steroid in rats and dogs respectively. In autoradiographic study, by applying radioactive HBP and HB to rat skin, it was revealed that more radioactive HBP was found in the malpighian layer than HB. From these results obtained, it was concluded that the characteristic features of HBP were 1) rapid percutaneous absorption, 2) strong affinity to steroid receptor, 3) efficient distribution in the viable epidermal layers and in the inflammatory cells.

4. Effects of an 8-Week Dietary Supplementation of Eicosapentaenoic Acid in Serum, PMNs and Epidermal Fatty Acids of Psoriatic Subjects. V. A. ZIBOH, C. MILLER, K. KRAGBALLE, K. COHEN, C. N. ELLIS, AND J. J. VOORHEES, Depts. of Dermatology, School of Medicine, University of California, Davis, CA, and School of Medicine, University of Michigan, Ann Arbor, MI.

It is known that skin from psoriatic patients generates excessive lipoxygenase products from arachidonic acid (AA). Since eicosapentaenoic acid (EPA) has previously been reported to inhibit platelet aggregation by competing with AA metabolism via the cyclooxygenase pathways, we were prompted to examine the effect of a diet rich in this FA on psoriasis to test whether dietary EPA could decrease generation of lipoxygenase products and alleviate the disease. Twelve adult patients with stable psoriasis vulgaris involving >10% of body surface were therefore fed a diet supplemented with 60–80 ml of fish oil (containing by weight 10% AA, 16.7% EPA and 10.6% docosahexaenoic acid (DCHA, 22:6, n3) per day for 8 weeks. Samples of serum, PMNs, skin (I and UN) were taken prior to dietary intake and at 2-weekly intervals up to 8 weeks. Total lipids from each specimen was extracted by chloroform/methanol (2:1) mixture, and transesterified overnight with methanolic-HCl to generate fatty acid methyl esters (FAMEs). The FAMEs were separated by gas liquid chromatography. Our data revealed striking uptakes of the n-3 fatty acid series (C20:5 and C22:6), and a decrease in the 18:2n6 acid into the various samples during the 8-week dietary period. Although there was only a negligible decrease in the level of 20:4n6 in these samples, patients with elevated ratios of 20:5n3/20:4n6 and 20:5n3/22:6n3 in involved (I) skin samples demonstrated clinical improvement of their psoriasis. These findings underscore the necessity of further studies into the role of dietary polyunsaturated fatty acid in the pathophysiology of psoriasis.

5. The Effect of 12-0-Tetradecanoylphorbol-13-acetate (TPA) on Membrane Phospholipid Metabolism of Human Keratinocytes in Culture. V. DELEO, S. SCHEIDE, H. HORLICK, AND D. HANSON, Columbia University, New York, NY.

The tumor promoter TPA stimulates proliferation in intact mouse epidermis and in murine keratinocytes in culture. While intact human epidermis responds similarly to this agent, growth of human keratinocytes in culture is inhibited by TPA. Since proliferation in the murine systems is thought to be mediated by TPA induced hydrolysis of arachidonic acid from membrane phospholipids (phospholipase A2 activation), we examined the effect of this agent on the phospholipid metabolism of human keratinocytes in culture. Human keratinocytes were grown in dishes and maintained in Keratinocyte Growth Medium with 10% fetal calf serum (KGM + 10). At confluency cells were labelled with either [3H]arachidonic acid (AA) or [3H]choline (Ch) 2μCi/ml in KGM + 10. After 24 hours, the cells were washed with KGM without serum (KGM + 0) and treated with KGM + 0 containing TPA in DMSO (1pg-lμg/ml) or DMSO alone (control). After 3 hours aliquots of media were assayed for release of radiolabel by liquid scintillation. Cells prelabeled with [3H]AA responded to very high concentrations (1μg/ml) of TPA with only minimal release of label—118.3 ± 5.5 percent of control ($\bar{x}$ ± SEM, N = 9). Conversely, cells prelabeled with [3H]Ch responded to the same concentration of TPA with a marked release of label—364 ± 25 percent of control (N = 3). These data indicate that while TPA is active in altering cellular membrane metabolism (Ch release), such perturbations do not result in the hydrolysis of AA from membrane phospholipids of these cells. These findings suggest that the lack of a TPA-induced proliferative response in human keratinocyte cultures is due to the inability of this agent to stimulate phospholipase A2 activity in such systems.

6. Serum Retinol-binding Protein Species in Human Epidermis. G. SIEGENTHALER, J-H. SAURAT, L. DIDIERJEAN, D. GRAND, AND R. HOTZ, Clinique de Dermatologie, Hôpital Cantonal Universitaire, Geneva, Switzerland.

Serum retinol-binding protein (sRBP) is the specific vector for the transport of retinol (R) to the target cells. It has been suggested that on binding to its membrane receptor, sRBP gives up R and becomes structurally modified: the apo-sRBP. Only holo-sRBP can interact with transthyretin (TTR). No information is available about the presence of the two forms holo-and apo-sRBP in the tissue and particularly in human epidermis. Besides its theoretical importance for membrane receptor concept this problem is of technical interest. When analysing the cellular retinoid-binding proteins (CRABP and CRBP) by radiobinding assay, sRBP can be a contaminant.

Normal (Heat Separated) Epidermis (NHSE) as well as Normal (NKS) and Psoriatic (PKS) Keratomised (280 μm) Skin samples were studied for sRBP species content with three different techniques. a) 100 K × g supernatants were incubated with ^{3}HR. Binding activities from gel filtration eluate were submitted to an affinity column with TTR coupled to Sepharose: no detectable (NHSE and NKS) or only trace amounts (PKS) of ^{3}HR-sRBP bound to the TTR were found. b) In vitro sRBP has practically the same binding affinity for R and retinoic acid (RA). Thus ^{3}HRA bound to sRBP can be displaced by an excess of R. Only less than 20% of the ^{3}HRA binding capacities in supernatant samples could be displaced by an excess of R, whereas more than 50% of the ^{3}HR binding activities could be displaced by RA, suggesting the presence of CRBP (F) rather than sRBP properties. c) Skin supernatant were submitted to PAGE and immunoblotted on nitrocellulose sheet, with a goat anti-human sRBP serum. Two bands, corresponding to the apo and holo form of the sRBP could be detected with the apo form being the major band.

These results demonstrate that apo-sRBP is the major soluble form of sRBP in human epidermis and suggest that sRBP does not significantly interfere with evaluation of CRABP and CRBP retinoid binding activities.

7. Vitamin A Binding Proteins in Human Epidermis, Dermis, Keratinocytes and Fibroblasts. R. E. GATES AND L. E. ALLRED, Dermal Research, Vanderbilt and V. A. Medical Center, Nashville, TN, and S. C. Johnson & Son, Racine, WI.

While vitamin A deficiency dramatically increases the keratinization of epidermis, striking effects on dermis have not been reported. A similar difference in sensitivity is observed *in vitro* where terminal differentiation of keratinocytes is inhibited by vitamin A, while morphology, saturation density or substrate adhesion of normal human fibroblasts are not affected. Levels of cytoplasmic vitamin A binding proteins were determined in dermis and epidermis separated by dispase treating human neonate foreskin and in fibroblasts and keratinocytes cultured from dermis and epidermis. The amount of [^{3}H]-retinol or [^{3}H]-retinoic acid specifically bound to cytosol proteins eluting in the 5- to 30-kDa range from 9 cm Biogel P-60 columns was used to quantitate relative amounts of cytoplasmic retinol binding protein (CRBP) or cytoplasmic retinoic acid binding protein (CRABP). Cytosols from untreated portions of skin had 75% of the CRBP or CRABP levels found in cytosols from dispase treated portions of the same skin, suggesting that dispase treatment improved homogenization of skin samples. CRABP levels were 5–10 fold higher than CRBP levels in whole skin, in separated epidermis, in keratinocytes and in fibroblasts. Expressed as pmoles per mg of cytosol protein, amounts of CRABP were 15.5 ± 5.2 (S.D.) for whole skin, 60.9 ± 13.6 for epidermis and 36.1 ± 7.0 for cultured keratinocytes. Amounts of CRABP in dermis and fibroblasts were much lower being 2.4 ± 1.9 and 9.2 ± 2.0, respectively. Increased amounts of CRABP in epidermis and keratinocytes compared to the amounts in dermis and fibroblasts correlate with the sensitivity of this cell type to vitamin A.

8. Effects of Oral Contraceptives or Pregnancy on Hormone Binding in Nevi. DARREL ELLIS, Dept. of Dermatology, Vanderbilt University and V.A. Medical Centers, Nashville, TN.

Nevi from women taking oral contraceptives or who are pregnant may undergo clinical changes in color and size. An increase in melanoma in patients taking oral contraceptives has also been reported. Using a fluorescent estrogen and progesterone binding technique, melanocytic nevi were removed from women who were pregnant, less than one month postpartum, or taking oral contraceptives, and assayed for estrogen and progesterone binding. Nevi from seven women taking oral contraceptives, three women who were pregnant, and two women who were less than one month postpartum were studied. Binding specificity was studied using unlabeled diethylstilbestrol for estrogen binding, unlabeled progesterone for progesterone binding, and L-DOPA. The assay detected specific estrogen and progesterone receptors as demonstrated by reversal or inhibition of binding by the appropriate unlabeled ligand. All nevi from these patients had markedly increased numbers of estrogen and progesterone binding cells. These findings contrast with acquired intradermal nevi from eleven women who were not pregnant or taking oral contraceptives, which had few or no detectable estrogen or progesterone binding cells. Conclusion: The increased hormone binding of nevi from pregnant women or women taking birth control pills may relate to the apparent clinical responsiveness of melanocytic nevi to hormonal changes, or to the increased incidence of melanoma in women taking oral contraceptives.

9. Localization of Diffusible Elements and Water in Skin. R. R. WARNER, M. C. MYERS, AND D. A. TAYLOR, Proctor and Gamble Co., Cincinnati, OH.

We have used analytical electron microscopy to explore element and water profiles in skin. The electron microprobe/analytical microscope can form an image of specimen ultrastructure at high magnification and determine, quantitatively, the distribution of elements associated with this ultrastructure with a resolution of several hundred angstroms. Diffusible substances can be maintained and analyzed in their *in-situ* locations with the use of ultra-cold sample preparative techniques. Water can also be quantitatively measured within submicron regions of a sample, providing a unique ability to measure, cell by cell, the water gradient across skin. Application of this technology to skin has shown: an approximately linear gradient of water across the stratum corneum (SC) with a large discontinuity in water concentration occurring at the SC-granulosum interface; a barrier to topically applied zinc salts occurring midway through the SC; large K, Na and Cl gradients starting midway through the SC and increasing to the skin surface; the virtual absence of K from the inner SC; and the virtual absence of P from the entire SC. This technology should provide new opportunities for correlative investigations of the structure and physiology of skin.

10. Skin Calcium-binding Protein in Human Epidermis. L. DIDIERJEAN, G. SIEGENTHALER, C. HEIZMANN*, AND J-H. SAURAT, Clinique de Dermatologie, Hôpital Cantonal Universitare, Geneva, Switzerland, and * Institut für Pharmakologie and Biochemie, Universität Zürich-Irchel, Zürich, Switzerland.

An antiserum directed against rat skin calcium-binding protein (SCaBP) specifically stained the basal cells of human epidermis. This antiserum was also used in a radioimmunoassay (JID *82*, 533 1984). The nature of this immunoreactive protein in human skin, however, has not been elucidated so far.

Therefore human epidermis (heat separated for 1 min at + 52°C and homogenized in 13 mM Tris-HCL plus PMSF 10 μg/ml) were separated on 1D SDS-PAGE and blotted onto nitrocellulose. Incubation with anti rat SCaBP serum revealed an immunoreaction only at MW of 12–13 Kd. Analysis of the same extract by 2D PAGE and immunoblotting showed a reaction with two components of identical MW of 12–13 Kd but different pI of 3.5 and 3.8, respectively.

Since SCaBP and parvalbumin have very similar properties and occasionally have shown a faint cross-reactivity under denaturing (SDS, but not under native conditions) it was necessary to determine if human epidermis contains one or the other or even both components. Separation of human epidermis extract by HPLC on reverse phase supports showed a protein peak at the position of purified rat SCaBP but not of parvalbumin, the latter which could not be detected in skin extracts. This result is in agreement with the immunohistochemical (J. Biol. Chem. *259*, 5189 (1984) and biochemical (PANS, *81* (1984) in press) results demonstrating the absence of parvalbumin in rat and human epidermis.

In slices of PAGE separated epidermis extract, the calcium binding property of the immunoreactive proteins was confirmed by competitive displacement of $^{45}Ca^{2+}$ binding peak by unlabeled Ca^{2+}.

The combined results demonstrate the presence of a SCaBP in human skin and further experiments have to be carried out to explore its precise role in this tissue.

11. Effect of Epidermal Growth Factor on Phosphatidylinositol Turnover of Pig Epidermis. TAKASHI AOYAGI, HIDEKO SUYA, AND YUSHO MIURA, Dept. of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan.

Our previous study suggests that epidermal growth factor (EGF) stimulated the release of arachidonic acid from membrane phospholipids through the activation of phospholipase A2. In order to elucidate the preceding process to enhance the activation of phospholipase A2, we studied the effect of EGF on phosphatidylinositol (PI) turnover of pig epidermis. PI turnover was monitored either by ^{32}Pi-incorporation into phosphatidic acid (PA) and PI, or by ^{3}H-inositol-incorporation into PI. ^{32}Pi-incorporation into PA and PI was significantly stimulated by EGF at 5 min. ^{3}H-inositol-incorporation into PI was also stimulated by EGF at 30 min and reached its maximum at 60 min. Stimulation by EGF on PI breakdown or PI resynthesis was independent of external calcium. These results indicate that EGF stimulated PI turnover (breakdown and resynthesis) of pig epidermis, although intermediate processes to lead to the activation of phospholipase A2 by EGF following PI turnover was not fully documented.

12. The Effects of Ionophore A23187 on the Cyclic-Nucleotide Levels. NAOKO KATO, HIDEKO SUYA, TAKASHI AOYAGI, YUSHO MIURA, AND KENNETH M. HALPRIN*, Dept. of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan, and *Dept. of Dermatology, University of Miami, Miami, FL.

Increased cyclic GMP levels and a refractory epinephrine induced receptor-adenylate cyclase-cyclic AMP response in psoriatic epidermis have been reported. Ionophore A23187 with Ca experimentally stimulates the release of arachidonic acid and the production of hydroxy fatty acids (HETE) through the activation of phospholipase A in various cell systems including epidermal keratinocytes. We investigated the effects of Ionophore and/or inhibitors of phospholipase A on the cyclic-nucleotides levels of pig epidermis with using floating system. Results are as follows: (1) Ionophore stimulated the accumulation of intracellular cyclic GMP. (2) Mepacrine and hydrocortisone, inhibitors of phospholipase A, inhibited the accumulation of cyclic GMP induced by Ionophore. (3) Epinephrine and prostaglandin E-adenylate cyclase-cyclic AMP response were decreased.

We reached the conclusion that activation of phospholipase A can reproduce both the cyclic GMP increase and the impaired epinephrine induced cyclic AMP response seen in psoriatic lesions. Inhibition of this enzyme may be of therapeutic benefit.

13. Muscarinic Effects on Corneal Epithelial DNA and RNA Polymerases. LYNN A. DRAKE AND ANN M. COLLEY, Depts. of Dermatology and Ophthalmology, Emory University School of Medicine, Atlanta, GA.

DNA and RNA polymerase activities in the purified nuclear fraction from cultured rabbit corneal epithelial cells were assayed over a range of substrate (labeled dTTP or UTP) concentrations using calf thymus DNA as template. Effects of carbamylcholine on polymerase activities were evaluated over a range of drug concentrations including those saturating for muscarinic receptors. For nuclear DNA polymerase in control incubations, Vmax = 18.9 ± 0.6 pmol/mg/protein/min and Km = 29 ± 6 μM. Control RNA polymerase had Vmax = 17.1 ± 2.3 pmol/mg/protein/min and Km = 654 ± 68 nM. Nuclei from carbamylcholine-pretreated cells had Vmax = 49.4 ± 2.4 pmol/mg protein/min, Km = 20 ± 3 μM for DNA polymerase and Vmax = 40.8 ± 0.7 pmol/mg protein/min, Km = 641 ± 28 nM for RNA polymerase. For nuclei from untreated cells assayed in the presence of the drug, Vmax = 83.1 ± 1.6 pmol/mg protein/min, Km = 8 ± 1 μM for DNA polymerase and Vmax = 42.1 ± 0.4 pmol/mg protein/min, Km = 671 ± 100 nM for RNA polymerase. Drug effects were blocked by atropine. Regression analysis of Hill plots for variation of DNA polymerase activity with carbamylcholine concentration revealed half-maximal activity at 8.2 μM carbamylcholine. Hill plots of RNA polymerase activity as a function of carbamylcholine concentration indicated half-maximal activity at 6.5 μM. Mechanisms by which carbamylcholine alters polymerase activities will be discussed in relation to effects of the drug on nuclear enzymes of cyclic nucleotide metabolism and on cyclic nucleotide-dependent protein phosphorylation.

14. Accumulation and Release of Proteases by the Isolated Human Eccrine Sweat Gland Following Pharmacological Stimulation in Vitro. H. YOKOZEKI AND K. SATO, Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

Human eccrine sweat contains a variety of proteolytic enzymes, however, their origin and physiological function remain unknown. Human eccrine sweat glands were isolated from biopsy skin specimens and incubated in Krebs' Ringer buffer (KRB at pH 7.4) at 37°C for up to 1 hour with or without addition of stimulants of sweat secretion (i.e., MCH, methacholine,and ISO, isoproterenol). Protease activity was determined in both the incubation medium (KRB) and glandular extracts (extracted first with KRB or Tris buffered saline (TBS) and subsequently with 2M KSCN) by incubating aliquots of the extracts with Kabi S-series substrates. Upon stimulation with MCH or ISO, sweat glands rapidly accumulate a variety of proteases but particularly S-2288 hydrolyzing enzymes (total serine proteases), peaking at 5 min in a dose-dependent and pharmacologically-specific manner. Release of proteases into the incubation bath takes place at a much slower but steady rate. Protease accumulation in the gland is inhibited by any agents or conditions which suppress sweat secretion, i.e. by inhibitors of sweat secretion, ouabain, furosemide, and in either Na^+- or Cl^--free medium. MCH-induced, but not ISO-induced, protease accumulation is completely inhibited in Ca^{2+}-free medium, as in sweat secretion by respective agonists. Isolated cystic fibrosis sweat glands, which lack

beta adrenergic responsiveness, responded normally to MCH but failed to respond to ISO. Protease accumulation may involve protein synthesis because it is inhibited by cyclohexamide and other inhibitors of protein synthesis. The intimate correlation between sweat secretion and protease accumulation suggests that it is in integral part of glandular function during sweat secretion.

15. The Mode of Action of Anthralin: Antirespiratory Activity and Subcellular Distribution in Cultured Human Keratinocytes. UWE REICHERT, RAINER SCHMIDT, AND BRAHAM SHROOT, Centre International de Recherches Dermatologiques (CIRD), Valbonne, France.

The molecular mode of action of the antipsoriatic drug anthralin is far from being understood. Besides its inactivating effect on enzymes, it has been shown to inhibit DNA replication and to destroy mitochondrial structure.

Using the transformed human keratinocyte line SV-K14 (from B. Lane, London) and determining the ^{14}C-CO_2-production from U-^{14}C-glutamine as a measure of respiratory activity we could demonstrate that cellular respiration is a more sensitive response to anthralin (ID50 = 1 μM) than 3H-thymidine incorporation (ID50 = 3 μM). Antimycin A, a known mitochondrial inhibitor behaves similarly. In contrast, mitomycin C, or 8-methoxypsoralen in combination with UVA, only weakly affect respiration, but reduce markedly TdR incorporation. Thus mitochondrial respiration is the primary target of anthralin action.

Kinetic studies indicate that a short incubation period of the cells with anthralin is sufficient to trigger the full biologic response, but that it takes some hours before the effect manifests itself (4 h after 10 min incubation with 3 μM). Subcellular distribution studies with 10-^{14}C-anthralin demonstrate that during the contact time of 10 min the label becomes mainly associated with the plasma membrane, but with increasing post-incubation time there is an accumulation (88% after 5 h) in an intracellular particulate fraction which consists essentially of mitochondria and lysosomes. Extraction of the cells and subcellular components by organic solvents and subsequent analysis reveal that there is no stable adduct formation of proteins or lipids with anthralin and that the radioactivity is essentially recovered in the form of oxidation products.

We conclude from our results that the main action of anthralin is membranotropic and that its oxidation products are preferably accumulated in the mitochondria giving rise to an inhibition of cellular respiration.

7:00 PM–9:00 PM RECEPTION — Organization of American States Building, 17th Street and Constitution Avenue

Friday, May 3, 1985

8:00 AM–10:30 AM

CONCURRENT SCIENTIFIC SESSIONS

CONCURRENT SESSION 2A: IMMMUNOLOGIC CYTOKINES

Presidential Ballroom

Hikaru Eto and Dan Sauder, Presiding

1. The Proliferative Response of Epidermal Cells (EC) to Con-A and IL-2 is Restricted Predominantly to Thy-1+ Cells. P. R. BERGSTRESER, J. L. NIXON-FULTON, AND R. E. TIGELAAR, Dept. of Dermatology, UTHSCD, Dallas, TX.

Bone marrow-derived dendritic cells bearing Thy-1 antigen (Thy-1 + dEC) are known to populate normal mouse epidermis. Recently, short-term cultures of EC, containing Thy-1+dEC, Ia+ cells, and keratinocytes, have been observed to proliferate in response to Con A and IL-2. Two strategies were used to identify the phenotype of these proliferating cells. In the first, trypsin-disaggregated EC from CBA mice were enriched to 30% Thy-1+ and 10% Ia+ cells by Isolymph centugation and then cultured in 96-well U-plates with or without 2.5 μg/ml Con A and 5 U/ml IL-2. Cultures were pulsed with 3H-thymidine for the final 24 hr of a 96 hr period and cytocentrifuged onto slides. Immunoperoxidase staining with monoclonal anti-Thy 1.2 or anti-Ia, followed by autoradiography permitted subsequent phenotyping of the proliferating cells. In unstimulated cultures, 0.5% of cells were in active DNA synthesis; 80% of these cells exhibited no detectable Ia or Thy-1 antigens. In cultures containing Con A and IL-2, 25% of cells were in active synthesis, with >90% expressing Thy-1. By contrast, no Ia+ cells were in active DNA synthesis. In the second protocol, EC were separated with the fluorescence activated cell sorter into Thy-1 enriched (96–99% Thy-1+) and Thy-1 depleted (>1% Thy-1+) populations. These cells were then cultured for 6 days with or without Con A and IL-2. Significant proliferation (stimulation index >10) was restricted to Thy-1 enriched cells; Thy-1 depleted cells gave stimulation indices invariably <2. We conclude that from these studies that the initial proliferative response to Con A and IL-2 is restricted predominantly to those epidermal cells which have been identified previously as Thy-1+dEC.

2. The Role of ETAF in Epidermial Cell Induced Con A-Driven T Cell Proliferation. ANGELIKA BINDER, GEORG STEINER, LAURA STINGL, KLAUS WOLFF, AND GEORG STINGL, Dept. of Dermatology I, University of Vienna, Vienna, Austria.

In a previous study, we have shown that murine epidermal cells (EC) can reconstitute Concanavalin A (Con A)-driven T cell proliferation. This function is dependent upon Ia-bearing Langerhans cells (LC). This requirement for LC cannot be replaced by keratinocyte-derived ETAF (IL-1-like cytokine)—a finding which, however, does not exclude the possibility that ETAF may represent a second critical signal for EC-induced T cell responses to Con A.

For this purpose EC were gently prefixed with paraformaldehyde (PFA, 0.005–2%; 1min) and then assayed for their capacity to reconstitute ConA-driven T cell proliferation. Results obtained revealed that highly purified lymph node T cells did not exhibit measurable proliferation when stimulated with either Con A (2.5–10 μg/ml), Con A + ETAF (50U/ml) or Con A + recombinant IL-2 (20U/ml). The response was restored by either untreated EC or LC-enriched EC, whereas PFA treatment of EC (which leaves the anti-Ia reactivity of LC unaltered) led to a dose-dependent reduction of the T cell response. Attempts to reconstitute the functional impairment of PFA-treated EC by the addition of exogenous ETAF showed that complete abolition of T cell proliferation by PFA-concentrations.

0.5% was not restored by addition of ETAF. When EC were treated with PFA-concentrations of 0.5%, the diminished T cell response was partly reconstituted by exogenous ETAF.

These findings indicate that ETAF/IL-1 may lead to an augmentation of accessory cell-dependent Con A-driven T cell proliferation but does not qualify as a critical second signal for the activation of resting T cells.

3. Cultured Keratinocytes Secrete a Substance Which Inhibits Allogeneic Lymphocyte Proliferation. BRIAN J. NICKOLOFF, T. Y. BASHAM, T. C. MERIGAN, AND V. B. MORHENN, Dept. of Dermatology and Div. of Infectious Diseases, Stanford University School of Medicine, Stanford, CA.

To better understand the interaction of human epidermal cells and lymphocytes *in vivo*, we have studied the effect of fresh and cultured epidermal cells on the proliferation of allogeneic peripheral blood mononuclear cells (PBML) *in vitro*. When cultured human keratinocytes (KCs) devoid of Langerhans cells (LCs) were induced to express HLA-DR by recombinant gamma interferon, and incubated with allogenic PBML, no proliferation of the PBML occurs.

By contrast, when fresh epidermal cell suspensions (containing 1-3% LCs) are coincubated with allogeneic PBML, ^{3}H-thymidine incorporation increase (40,000 cpm over background) after 7 days (5×10^4 epidermal cells, 5×10^4 lymphocytes, 2 μCi/ml ^{3}H-thymidine). To explain this difference between fresh and cultured cells, we investigated the possible production of an inhibitory factor by cultured untreated KCs.

When approximately 5×10^4 KCs growing on collagen-coated microtiter wells are incubated with 5×10^4 stimulator plus responder PBMLs, the expected mixed lymphocyte reaction (MLR), which in the absence of KCs produced 25,000 cpm, was reduced by 90%. Also, 24 hour keratinocyte conditioned medium inhibits the MLR. The ability of cultured KCs to inhibit the MLR could not be reversed by 1. preincubation of the KCs with indomethacin (3×10^{-6} M), 2. addition of anti-alpha, beta and gamma interferon antibodies, 3. overnight dialysis. Thus it appears that KCs produce factors which stimulate (ETAF) and inhibit lymphocyte proliferation.

4. Intracellular (or Membrane Bound) IL1 is Present in Normal Human Epidermis. C. HAUSER, J-H. SAURAT, AND J-M. DAYER*, Clinique de Dermatologie and * Division d'Immunologie et

d'Allergologie, Département de Médecine, Hôpital Cantonal Universitaire, Geneva, Switzerland.

We previously found that factors which stimulate PGE2 and collagenase of dermal fibroblasts are present in cell-lysates and conditioned media of cultured human epidermal cells (J.I.D. 82: 564, 1984). These factors share some physico-chemical characteristics of IL1. We have investigated the existence of similar factors in ex vivo freshly prepared epidermis. Suction blister roots and heat separated epidermis obtained from cosmetic surgery skin were used as starting material to search for PGE2- and collagenase-stimulating activities. Suction blister roofs raised on healthy young individuals were cut off, extracted, dialyzed and tested on dermal fibroblasts for the stimulation of PGE2 and collagenase. The results showed that suction blister epidermis extracts of 5/5 individuals produced PGE2- and collagenease-stimulating activities (PGE2: 34.2–108.3 ng/ml, collagenase: 3.04–4.2 units/ml: medium alone PGE2: < 1.0 ng/ml: collagenase: 0.2 units/ml). In addition, an extract from heat separated epidermis (52°C, 1 min) stimulated both fibroblast PGE2 and collagenase. Molecular seize exclusion chromatography (Ultrogel AcA 54) of heat separated epidermis yielded peaks corresponding to MW of ~ 15 and ~ 35 Kd.

These results suggest that normal human epidermis contains intracellular or membrane bound factors which stimulate PGE2 and collagenase of dermal fibroblasts and which resemble IL1.

5. Macrophage-derived Interleukin 1 Activity in Atopic Dermatitis. MASAKO MIZOGUCHI, SHUICHI FURUSAWA, SHOKO OKITSU*, AND KAZUYA YOSHINO*, Depts. of Dermatology and * Pediatrics, Teikyo University School of Medicine, Tokyo, Japan.

Patients with atopic dermatitis (AD) exhibit skin infection susceptibility and defective delayed type hypersensitivity. In order to study the relationship between these immune abnormality and macrophage function, we studied interleukin 1 (IL 1) which is a macrophage-derived protein and modulates many immune responses, such as host defense against infection or T cell proliferation through the induction of interleukin 2. Culture supernatants of lipopolysaccharide-stimulated peripheral blood monocytes (macrophages) from 12 AD patients and 10 age matched normal controls were used as IL 1 source. High pressure liquid chromotography-purified IL 1 (culture supernatant) were also used. IL 1 activity was assayed by adding the supernatants to cultures of C3H/He.J mouse thymocytes (1.5×10^6 cells/well) with phytohemagglutinin (PHA) for 72 hrs. After a 6-hr pulse with (^{3}H) thymidine, cells were harvested and counted for incorporation of radioactivity. The mean value of IL 1 activity (stimulation index (SI) 9.3 ± 2.7) from macrophages of AD patients was significantly lower than that (SI = 12.1 ± 2.9) of normal controls. The patients who had low IL 1 activity showed defective lymphocyte proliferation in vitro in the presence of PHA or other mitogens. However, there was no significant correlation between IL 1 activity and the severity of AD. Nevertheless, low IL 1 activity suggests defective macrophage function and might account for some immune reactions in AD.

6. *In Vivo* Chemotactic Activity of Epidermal Cell-derived Thymocyte Activating Factor (ETAF) in the Mouse. R. D. GRANSTEIN, R. J. MARGOLIS, AND D. N. SAUDER*, Dept. of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, MA, and * Div. of Dermatology, McMaster University, Hamilton, Ontario, Canada.

Keratinocytes secrete a cytokine termed ETAF that has a multiplicity of functions including *in vitro* chemotactic activity. In this study *in vivo* chemotactic activity of ETAF was assessed. ETAF was prepared from culture supernatant of normal keratinocytes and purified by ultrafiltration and Sephadex G100 chromatography. In other studies, ETAF was further purified by high performance liquid chromatography. 0.05 ml of ETAF in phosphate buffered saline (PBS) was injected subcutaneously (SC) into the left hind footpad of BALB/c mice while the right was injected with PBS as a control. Nontreated mice served as an additional control. Mice were sacrificed at various time intervals and footpad skin was processed for routine light microscopy. Inflammatory cells infiltrating the dermis and subcutaneous tissue were counted in 4 areas in each tissue section. Compared to controls (which were not significantly different from uninjected mice at any time point), a significant accumulation of neutrophils was seen (ETAF vs media) at 1 h (561 cells/mm^2 ± 148 SEM vs 89 ± 15, $p = 0.041$) with peak accumulation at 8 hr (3,138 ± 133 vs 122 ± 15, $p < 0.001$) and disappearance by 72 hr (76 ± 43 vs 97 ± 25, $p = 0.800$). As early as 1 hr margination of neutrophils within dermal vessels was apparent. Significant mononuclear cell infiltration was also present with similar kinetics, although total accumulation was less than that of neutrophils. These effects were also seen in C3H/HeJ mice, thus excluding endotoxin contamination as a cause of these findings. The results of this study demonstrate the *in vivo* chemotactic properties of ETAF for neutrophils and provide an important new model for localized inflammatory processes in the skin.

7. Human Epidermal Cell Production of a Natural Killer Cell Activity Augmenting Factor (ENKAF) Which is Distinct from ETAF/IL1, IL2 and IFN. MICHAEL MICKSCHE, MICHEL COLOT, ANDREAS KÖCK, AND THOMAS A. LUGER, Dept. of Dermatology II and Institute of Applied and Experimental Oncology, University of Vienna, Vienna, Austria.

Increasing evidence suggests that natural killer (NK) cells play an important role in host defense mechanisms. Epidermal cells (EC) recently have been demonstrated to release an Interleukin 1 (IL1) like immunoregulating cytokine (ETAF). Because IL1 together with Interleukin (2(IL2) or Interferon (IFN) synergistically enhances NK cell activity, the effect of ETAF containing EC and squamous carcinoma cell line (SCC) supernatants on NK cell activity was investigated. Large granular lymphocytes (LGL, NK cells) were purified by discontinuous percoll gradient centrifugation. Using a ^{51}Cr-release assay with K 562 erythroleukemia cells as targets, EC and SCC derived supernatants itself were able to augmented NK cell activity. Neither EC nor SCC supernatants contained IL2 or IFN activity. In contrast IL1 containing supernatants did not affect NK cell activity. For biochemical characterization high pressure liquid chromatography (HPLC) was performed. ENKAF like ETAF has a molecular weight between 25 and 10 Kd. Upon reverse phase HPLC ENKAF eluted separated from ETAF. Active cell metabolism of lymphocytes was necessary for ENKAF induced augmentation of NK cell activity. The number of LGL binding to K 562 was not affected by ENKAF, but the frequency of dead conjugated target cells was increased by pretreatment with ENKAF. In contrast to ETAF, ENKAF was not chemotactic for polymorphonuclear granulocytes, although it enhanced the motility of LGL. Additional incubation of LGL with ETAF did not further increase ENKAF mediated augmentation of NK activity. These findings demonstrate that EC release an additional mediator which augments NK cell activity, but is distinct from ETAF/IL1, IL2 and IFN and thereby may mediate the activity of cytotoxic cells.

8. Immunological Properties of an Epidermal Cell Derived Lymphocyte Differentiating Factor (ELDIF). J. F. NICOLAS, D. KAISERLIAN, M. FAURE, I. GIGLI, AND J. THIVOLET, Div. of Dermatology, Hôpital E. Herriott, Lyon, France, Div. of Immunology, Hôpital Necker, Paris, France, and Div. of Dermatology, University of California, San Diego, CA.

We have recently described a lymphocyte differentiating factor (ELDIF), produced by cultured human keratinocytes. ELDIF is distinct from prostaglandins, ETAF, the well defined thymic hormones thymulin, thymopoietin, and thymosin α1. It does not display any IL1, IL2 and IL3 activity. ELDIF is responsible, in a rosette bioassay, for the induction of the Thy 1 membrane antigen on T cell precursors. In order to study the effect of ELDIF on lymphocyte proliferative responses we concentrated ELDIF activity and eliminated other soluble factors known to exhibit immunological properties, by passing a 14 day old epidermal cell culture (ECC) supernatant through a Bio-Gel P2 column. The effect of both, crude ECC supernatants and semi-purified ELDIF was tested in a lectin induced lymphocyte proliferative assay. In these experiments, ELDIF greatly inhibited the proliferative response of spleen lymphocytes to Con A, PHA and LPS. This inhibition was dose dependent and not due to a toxic effect, since the viability of the lymphocytes was not affected by ELDIF treatment. In association with the inhibitory effect of ELDIF on lymphocyte proliferation, a marked decrease in IL2 production by Con A stimulated spleen cells was noted.

Thus, ELDIF, which displays T lymphocyte maturation activity, also exhibits a potent suppression of lymphocyte proliferative responses. Such results suggest that ELDIF may play an important role in the regulation of cutaneous inflammatory reactions.

9. Proliferating Human Keratinocytes Contain Interferon in the Absence of Viral Infection. M. YAAR, A. V. PALLERONI*, AND B. A. GILCHREST, USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, and * Dept. of Experimental and Applied Biology, Hoffmann-La Roche, Nutley, NJ.

Like many other cells, human keratinocytes (HK) are known to

make interferon (IF) in response to viral infection and to be reversibly growth inhibited by exogenous IF in vitro. In order to determine whether IF might participate in HK growth regulation in normal skin, studies were undertaken to detect epidermal IF in the absence of viral infection or other recognized exogenous stimulus. Cross sections of 15 normal human skin specimens, oral mucosa, and stratified epidermal keratinocyte cultures grown in a serum-free system were incubated with rabbit serum containing antibodies to cloned human alpha-A-IF, then with fluorescin tagged goat anti-rabbit IgG. In all instances, strong positive staining was uniformly present in the basal cell layer with occasional weaker staining in the first few suprabasilar layers, while control specimens incubated with pre-immune serum were negative. To assess the prevalance of IF in body tissues, healthy BALB/cByJ mice were sacrificed and tissue sections processed as above. Murine skin and esophagus showed basal epidermal staining identical to that of human skin, while non-mitotic tissues including brain, kidney, liver, and muscle were negative. These experiments establish that IF is present in the proliferative compartment of normal epidermis in vivo and in vitro and suggest that non-virally infected HK may synthesize IF. The combined data are consistent with a role for IF as a physiologic negative growth modulator or chalone in human skin.

10. Gamma-Interferon (γ-IF) Induces HLA-DR Antigen Expression by Human Dermal Microvascular Endothelial Cells (DMVEC) *In Vitro*. R. D. SONTHEIMER, Dept. of Dermatology, UTHSCD/SWMS,, Dallas, TX.

Cultured EC from large vessels (e.g. umbilical vein, aorta) of humans and experimental animals have been shown to be capable of carrying out several immunoregulatory functions. Since there are a number of known physiologic differences between EC from large and small vessels it is not safe to assume that DMVEC are endowed with the same immunoregulatory qualities possesed by large vessel EC. We have therefore begun to explore the immunological characteristics of human DMVEC. Confluent human DMVEC cultures were established from newborn foreskin using a modification of the Davison, et al technique (JID 75:316, 1980). First passage cells, confirmed to be EC by demonstration of factor VIII production, were examined by an indirect immunofluorescence technique for expression of two antigens (HLA-DR and Thy-1) that have been shown to be expressed by dermal microvessels *in vivo*. Neither antigen was found to be present on DMVEC grown under standard culture conditions. However, when DMVEC were cultured in the presence of γ-IF (250 μ/ml for 4 days), a subpopulation was found to express HLA-DR antigen in a granular cytoplasmic distribution. γ-IF failed to induce Thy-1 antigen expression. Studies are underway to determine if γ-IF treated DMVEC express other HLA-D region products (DC, SB) and are capable of stimulating allogenic T lymphocytes. These studies have shown that γ-IF can induce cultured human DMVEC to express one of the HLA-D region gene products that is required for certain immunologically relevant cell-cell interactions. Human DMVEC can now be examined for those immunoregulatory features (e.g., accessory cell and antigen presenting cell function) that are possessed by some other HLA-DR antigen bearing immunocompetent cells.

11. Lymphocyte Products Induce Production of Epidermal Cell Proliferation Factors(s) from Bone Marrow Cells. I. KATAYAMA, K. HASHIMOTO, AND K. NISHIOKA, Dept. of Dermatology, Osaka University School of Medicine, Osaka, Japan.

Elevated mitotic index of the basal cells and/or epidermal hyperplasia are observed in contact sensitivity skin reaction. An intradermal injection of lymphocyte products or compound 48/80 could induce increased epidermal mitogenesis. The aim of this study is to analyze responsible factors which modulate epidermal cell proliferation in vitro. Bone marrow (BM) cells from Balb/c mice were cultured in the presence of Con A sup (culture supernatant of Con A stimulated spleen cells of Balb/c mice). The culture supernatant of BM cells was added to PAM cell (5,000/ well of a microtest plate, Falcon #3072). The culture was continued for 48 hr. 0.2 μCi/well of ^{3}H thymidine was pulsed 24 hr before harvest. Dose dependent epidermal cell proliferating activity (EPF) was noted in the culture sup of BM cells. Con A sup, Con A itself or culture sup of BM cells without Con A sup did not show any EPF. EPF showed good correlation with the content of histamine and numbers of mast cell-like cells in the culture. EPF inducing factor in Con A sup was excluded from DE 52 and had a molecular weight between 30 and 40kD. It did not contain TCGF activity. Thus lymphocyte products with molecular weight of 30–40kD stimulate mast cell-like cells of BM to generate factor(s) responsible for epidermal proliferation.

12. Hemopoietic Colony Stimulating Activity Derived from Human Keratinocytes. J. A. DENBURG AND D. N. SAUDER, Dept. of Medicine, McMaster University, Hamilton, Ontario, Canada.

Human keratinocytes are known to produce a variety of soluble mediators termed cytokines, including epidermal cell thymocyte activating factor (ETAF). ETAF has, among its many functions, chemotactic activity for human leukocytes. In this study we investigated the possibility that the epidermis influences growth and differentiation of hemopoietic stem cells. ETAF containing supernatants from normal keratinocytes or from the skin squamous cell carcinoma line, COLO-16, were harvested after 5 days of culture and purified sequentially by ultrafiltration, Sephadex G-100 chromatography, and high performance liquid chromatography using a Synchropak AX 300 ion exchange column, followed by a TSK 4000 size exclusion column. The effect of ETAF containing supernatants (800 units/ml) on normal human peripheral blood hemopoietic progenitors was assayed at 14 days in methylcellulose cultures by scoring granulocyte colonies under inverted microscopy. ETAF from COLO-16 had a marked stimulating effect on colony formation: at a 1% v/v concentration, significant stimulation of colony formation was seen (69 ± 17 total colonies vs 14 ± 2.3 for medium control; $p < .01$). This effect was also seen at concentrations as low as 0.1% v/v. A similar effect was seen in the presence of normalkeratinocyte-derived ETAF. The derivation of hemopoietic growth factors from human keratinocytes suggests that one mechanism underlying the accumulation of inflammatory and perhaps neoplastic cells in the skin may be related to differentiation *in situ* of progenitor cells. Thus, within the epidermis, cytokines are produced that not only influence ingress of inflammatory cells to the skin site, but also may affect their differentiation *in situ*.

CONCURRENT SESSION 2B: CELL CULTURE

Congressional

Yukio Kitano and Edward O'Keefe, Presiding

1. Human Prokeratinocytes *In Vitro*: Senescence Versus Terminal Differentiation. MARK R. PITTELKOW, JOHN J. WILLE, JR., AND ROBERT E. SCOTT, Depts. of Dermatology and Cell Biology, Mayo Clinic/Foundation, Rochester, MN.

It has been suggested that *in vitro* senescence is equivalent to terminal differentiation but this concept has never been firmly demonstrated. We present evidence that human prokeratinocytes (HPK) senesce *in vitro* without expression of markers of keratinocyte terminal differentiation. Utilizing serum-free culture with MCDB 153 medium, growth factors and supplements, HPK from neonatal and adult skin are capable of >50 and >30 population doublings, respectively, and eventually arrest in the G_1 cell cycle phase (1). Serially passaged cells assume progressively larger size. Upon quiescence, these senescent cells attain a characteristic morphology and ultrastructural examination reveals scant, disorganized tonofilaments, accumulation of large osmophilic lamellar bodies and vesicular structures. Histochemical studies show that these cells contain neutral fats and phospholipids. Senescent cells fail to react with differentiation specific stains and monoclonal antibody to differentiation related antigen. However, such cells readily express these markers of terminal differentiation when cultured in growth factor deficient medium containing 2 mM calcium. Thus, senescent cells arrested in the $G_{1/0}$ state have been demonstrated to posses at least two alternative potentialities, quiescence without propensity toward terminal differentiation and a capacity to respond to inducers of terminal differentiation. Therefore, senescence of HPK is not inherently equivalent to terminal differentiation. (1) Wille, J. J., et al., J. Cell. Physiol. 121, 31–44, 1984.

2. Keratinocyte Culture Confluence Results in Suppression of Prostaglandin (PG) Synthesis and [^{3}H]-Thymidine (Tdr) Incorporation. A. PENTLAND AND P. NEEDLEMAN, Depts. of Dermatology and Pharmacology, Washington University Medical School, St. Louis, MO.

Keratinocytes isolated from adult human skin were plated at densities of 5, 2.5 .75 and .25 × 10^5 cells/cm^2. These plating densities result in 2 confluent (C) culture groups & 2 non-confluent (NC) culture groups. In 3 to 5 day old cultures, PGE_2/μg protein/ml of medium was determined by RIA after 4 or 24 hours incubation. A 20× increase in PGE_2 synthesis was found in NC cultures relative to C cultures. Cellular lipoxygenase and cyclooxygenase activities were studied by incubating [^{14}C]-arachidonic acid (AA) with the cultures and analyzed by thin layer chromatography. 10 to 15% of the [^{14}C]-AA was metabolized to product in one hour. C and NC cultures synthesized the same products

of AA, but PGE_2 synthesized by NC cultures represented up to 50% of the products formed, a 3× increase over C cultures. Dose-response data using cold AA indicated that the E C_{50} in C & NC cultures was the same, but C cultures produced only one twentieth as much PGE_2 as NC cultures. This decreased PGE_2 synthesis was associated with a 75% decrease in proliferation in C cultures as measured by [^{3}H]-Tdr incorporation into DNA. Autoradiography of [^{3}H]-Tdr labelled cells grown on coverslips confirmed this result. The proliferation of NC cultures but not C cultures was inhibitable by addition of 10 μg/ml indomethacin. These data show that NC cultures have an increased PG synthetic capacity which modulates their rate of proliferation until confluence is reached. This modulation may be important in wound healing or epidermal disease states.

3. Epidermal Cell Mediators of Connective Tissue Metabolism: Modulation by Cell Culture Conditions. A. D. HERNANDEZ, G. P. STRICKLIN, E. A. EVERS, AND M. E. CATES, VA Medical Center and University of Tennessee Center for the Health Sciences, Memphis, TN.

Epidermal-derived factors have been identified which stimulate dermal fibroblast proliferation and synthesis of glycosaminoglycans, collagenase, and collagenase inhibitor. This study uses a murine epidermal cell line, PAM 212, as a model system. Conditioned, serum-free medium from proliferating, confluent, and disrupted-confluent cultures of these cells was concentrated 30X, then subjected to gel filtration chromatography. The resultant fractions were diluted in fresh medium and placed over confluent cultures of normal human dermal fibroblasts or mouse thymocytes. Assays were then performed for human collagenase, collagenase inhibitor, and thymocyte proliferation. Subconfluent epidermal cells produced factor(s) of approximately 20 Kd which stimulated all three activities. However, higher weight fractions (55 Kd) were also present which stimulated the expression of collagenase and its inhibitor, but did not affect thymocyte proliferation. In contrast, confluent epidermal cells expressed only the high weight stimulators. Mechanical disruption significantly increased the expression of the high weight stimulators and the 20 Kd material which stimulated thymocyte proliferation. However, relatively less stimulation of collagenase and collagenase inhibitor expression was seen. We conclude that epidermal cell directed regulation of fibroblast functions is altered by culture conditions and that physical disruption is not equivalent to subconfluence. Furthermore, regulation of fibroblast expression of collagenase and its inhibitor is mediated by factors other than the previously described ETAF.

4. Characterization of a Hybridized Human Epidermal Continuous Cell Line. BRIAN BERMAN AND BONNIE SMITH, Depts. of Dermatology, University of California, Davis, CA, and Veterans Administration Medical Center, Martinez, CA.

Human epidermal cells isolated from adult breast skin were fused in suspension to cells of a HGPRTase-deficient human fibrosarcoma (T-1080) cell line using 45% polyethylene glycol 1000. Hybrids were selected in RPMI-1640 medium containing aminopterin lethal to parental T-1080. Approximately 1% of the hybridized cells expressed cell surface HLA-DR antigenicity. Panning on goat anti-mouse IgG coated plates for such DR bearing hybrids having bound monoclonal anti-DR IgG, yielded a population of cells (H12) capable of 100% expression of DR following incubation with recombinant γ-interferon (γ-IFN; 1000 U/ml). γ-IFN induction of DR expression was abrogated by actinomycin D or cycloheximide and markedly inhibited when H12 cells were preincubated with amcinonide. 1% of H12 cells express T200 antigens, and 5% are induced to express Fc receptors following exposure to γ-IFN. H12 cells were devoid of DOPA oxidase activity, melanosomes,keratin filaments, desmosomes or classical Birbeck granules and staining with monoclonal antibodies failed to reveal the presence of keratin or Factor VIII. H12 cells lacked the surface antigens detected by OKT4, OKT8, OKT11 and OKT6 antibodies. T6 antigenicity was not induced following exposure of H12 cells to γ-IFN, thymopoietin pentapeptide, serum thymic factor, thymosin alpha-1 fragment 25–28, colony stimulating factor, DMSO, cAMP, cGMP, calcitriol, 7-dehydrocholesterol, cholecalciferol, human epidermis, epidermal cells or their culture medium. The epidermal cell type giving rise to these cells needs further identification, yet the availability of a continuous line of cells on which DR expression is rapidly induced will aid in the elucidation of the role of γ-IFN in such induction.

5. Proportion of Cycling Cells in Different Morphologically Defined Populations of Human Epidermal Basal Cells. ROBERT A. BRIGGAMAN, Dept. of Dermatology, University of North Carolina School of Medicine, Chapel Hill, NC.

The purpose of this study was to determine the proportion of cycling cells in morphologically defined populations of human epidermal basal cells. The basal cell layer is composed of serrated cells which have a distinctive serrated interface at the epidermal-dermal junction and are present predominantly overlying dermal papillae while non-serrated basal cells have a flat epidermal-dermal junction and are located predominantly in the rete ridge areas (Lavker and Sun Science 215:1239, 1982). Normal human thigh skin was grafted to nude mice for 6–8 weeks after which tritiated thymidine was continuously administered by minipump infusion (Alzet Model #2002) over a 14-day period. Grafts were harvested and autoradiographs prepared using 1 micron plastic embedded sections. Basal cells were scored as labeled or unlabeled and morphologically as serrated, non-serrated or undetermined. 48% ± 7 (SD) of the total basal cells were labeled indicating that only a portion of epidermal basal cells are actively cycling. 34% ± 6 (SD) of serrated cells were labeled whereas 63% ± 10 (SD) of non-serrated cells were labeled. A significantly higher proportion of non-serrated cells are cycling compared to the serrated cells. However, the distinctive morphologic differences among epidermal basal cells do not define an exclusive proliferative cell population. These studies indicate that human epidermal basal cells are morphologically and functionally heterogeneous.

6. Culture of Epidermal Basal Cells on the Fibrin Gel Prepared from Platelet-rich Plasma. HAJIME TAKAHASHI, YASUMI KOGANEI, AND NOBUYUKI SHIOYA, Dept. of Plastic Surgery, Kitasato University School of Medicine, Sagamihara, Japan.

There have been many attempts to improve growth and proliferative conditions of epidermal basal cell culture. In general, it is known that, in the process of wound healing, cells grow and proliferate on the fibrin as a scaffold. Consequently, we made a fibrin gel derived from platelet-rich plasma. Then, we cultured these cells on the fibrin gel and compared it with another substrate, type IV collagen from human placenta, because the basement membrane is composed of this collagen, and plain dish. Epidermal cells were collected as usual using dispase and trypsin from inbred male Fischer newborn rats. The fibrin gel dish was prepared as follows. After blood was drawn with 2.5 mM EGTA, it was centrifuged at 1,200G for 10 minutes. Only the serum, containing platelets, was collected and added to 100mM $CaCl_2$. This, in culture dish, was incubated to a gel state. The rate of keratinocytes attaching on the platelet-rich fibrin gel was about 5.7 fold greater than on the plain dish, but was less than that on type IV collagen. On the ninth day, the attached cell numbers of the former were also greater than those of the latter, and made a wide spreaded epidermal sheet with clear pavement pattern, but less of type IV collagen. These findings indicate that, in the case of epidermal cell culture, it is highly probable that fibrin gel prepared from platelet-rich plasma would be a nutritional and a good natural culture substrate.

7. Association of Plasminogen Activator with Epidermal Cell Differentiation and Migration. SHINJI MORIOKA, PAMELA J. JENSEN, AND GERALD S. LAZARUS, Dept. of Dermatology, University of Pennsylvania, Philadelphia, PA.

Plasminogen activator (PA) has been associated with cell differentiation and fibroblast migration. To examine the role of PA in keratinocytes, we have localized PA in neonatal foreskin keratinocyte cultures, using a rabbit antibody against human urinary PA (urokinase). Cells were fixed in glutaraldehyde, permeabilized with saponin, and then incubated with immune or non-immune IgG. Specific staining for PA was detected with the avidin-biotin peroxidase complex technique. Primary keratinocyte cultures revealed 2 different localization patterns for PA. First, mature squames spontaneously shed into the culture medium or on the surface of the colony were homogenously and heavily stained. Differentiated cells thus appear to have relatively elevated PA levels. The second pattern was observed in cells at the periphery of subconfluent colonies, which had cytoplasmic granular staining. If confluent cultures were wounded by cutting with a blade, the cells at the wounded edges developed similar dark staining with anti-urokinase antibody within 12 hours. PA induction in wounded cultures was not inhibited by mitomycin C at a concentration (30 μg/ml) that blocked DNA synthesis by 90%. By contrast, cytochalasin B (50 μM) inhibited both cell migration and PA induction. These data indicate that PA may play a role in both epidermal differentiation and epidermal cell movement.

8. Divalent Cation Dependence of Epidermal Cell (EC) Spreading in Primary Culture. KURT S. STENN AND NOLAN G. CORE, Dept. of Dermatology and Laboratory Medicine, Yale University School of Medicine, New Haven, CT.

Understanding the mechanism of EC spreading is central to wound closure therapy. To that end the importance of divalent cations to the spreading of dissociated guinea pig EC was studied. Early studies showed that while divalent-cation-free Dulbecco's modified Eagle's medium (DME) plus fetal calf serum (FCS) supported no spreading, addition of Mg^{++} (2mM) yielded maximal spreading; in contrast, addition of Ca^{++} yielded much less spreading. Spreading assayed in a simple salt solution (25 mM Tris, 154 mM NaCl, 5 mM KCl pH 7.4) (SKT) plus FCS and a series of different cations (chlorides of Ca, Mg, Mn, Co, Zn, Cd, Ni, La) showed that only Mg^{++}, Mn^{++} and to a much less degree Ca^{++} suported EC spreading. Mg^{++} in SKT supported spreading only if serum were present. Mg^{++} supported no spreading in SKT plus BSA. In contrast Mn^{++} supported spreading in SKT in the absence of serum in the presence of BSA, a protein otherwise inactive. EC spreading in SKT plus FCS and Mn^{++} occurred at a lower cation concentration than SKT plus FCS plus Mg^{++}. While EC spread rapidly in Mn^{++}-media, they uplifted by 24 hrs. suggesting toxicity. In Mg^{++}-media EC showed persistent maximal spreading beyond 24 hrs. In contrast to EC, dermal fibroblasts spread in SKT plus Mn^{++} in the absence of any protein supplement.

These studies indicate that specific cations are crucial to EC spreading, that Mg is the active divalent cation in DME + FCS, and that Mn^{++} can support early EC spreading in the absence of specific spreading proteins.

9. Calcium Elevation Increases Confluent Density of Cultured Human Fibroblasts. F. C. PRAEGER AND B. A. GILCHREST, USDA Human Nutrition Research Center on Aging at Tufts University. Boston, MA.

Increased extracellular calcium concentration ($[CaCl_2]$) is known to increase confluent density for an established mouse fibroblast line and for WI 38 fibroblasts. In order to examine the effect of elevated $[CaCl_2]$ on human dermal fibroblasts, paired cultures of newborn foreskin fibroblasts (NFF) were seeded in standard nutrient medium containing 1.8 mM $CaCl_2$ and fetal bovine serum (FBS), then maintained undisturbed at 37°C in 5% CO_2 until confluent (7 days). Compared to controls in basal medium, NFF exposed to 1.5–5.0 mM additional $CaCl_2$ had a 150–400% increased cell yield. Increase in confluent density was maintained through 11–15 passages by NFF continuously exposed to elevated $[CaCl_2]$ but was lost in late passage. The effect was independent of FBS concentration and confluent density of cells exposed to elevated $[CaCl_2]$ with 5% FBS was greter than that for cells in basal medium with 20% FBS. All cultures responded with further growth to refeeding, but cultures refed medium with elevated $[CaCl_2]$ were stimulated 7–9 fold more than cultures refed basal medium ($p < .01$). Effect of donor age was studied by comparing NFF to fibroblasts derived from inner arm biopsies of 3 young (23–27 yr) and 4 old (66–83 yr) adults. Compared to controls, an additional 2 mM $CaCl_2$ increased cell yield $353 \pm 22\%$ ($p < .01$) for newborns, $184 \pm 12\%$ ($p < .01$) for young adults but $20 \pm 6\%$ for old adults. These data indicate that elevated $[CaCl_2]$ decreases density dependent growth inhibition of dermal fibroblasts in vitro and that this effect is donor age dependent. Elevated calcium may enhance fibroblast responsiveness to serum mitogens and/or mimic membrane effects of other growth regulatory molecules.

10. Cultured Mouse Preputial Gland Sebocytes Retain Testosterone Metabolic Activity In Vitro. JOEL L. BRIND, DORI POCIUS, SHIN-WEI KUO, AND NORMAN ORENTREICH, Orentreich Foundation for the Advancement of Science, Inc., New York, NY.

Previous *in vitro* sebaceous gland studies have generally relied upon preparations with contaminating tissues such as hair and epidermis also present. Isolated mouse preputial gland cells have provided a purely sebaceous cell model, but it is limited to short-term studies of freshly isolated cells. We have now put into tisue culture sub-populations of pure, normal mouse sebocytes in different stages of maturation.

Mature male CF-1 mouse preputial glands were disaggregated in trypsin and collagenase, the cells separated according to density (sebum content) on a Metrizamide step gradient, and five sub-populations were harvested and put into separate culture flasks. The cells were maintained in vitamin and amino acid enriched Eagle's Minimum Essential Medium and 10% fetal bovine serum in a 5% CO_2 in air atmosphere. Even the cells closest to maturity were able to attach and divide and although slow-growing, were stable in culture for at least three weeks.

Actively differentiating sub-populations of sebocytes demonstrated hydroxysteroid 5-alpha-reductase and 17-beta-dehydrogenase activities when incubated with 2×10^{-6} M [^{14}C]testosterone. Rates of enzyme activity after two weeks in culture were similar to those found in freshly isolated cells.

We are currently studying other markers of sebaceous differentiation (e.g., wax ester synthesis) in these cultured cells. The culture of purely sebaceous cells should prove a useful new tool in elucidating the mechanisms of sebaceous gland differentiation and regulation by endogenous factors and drugs.

11. High Yield Purification of Plasma Membranes from Cultured Human Keratinocytes. R. SCHMIDT AND UWE REICHERT, Centre International de Recherches Dermatologiques (CIRD), Valbonne, France.

The study of drug induced changes at the plasma membrane level in epidermal cells is limited by the difficulty in obtaining these membranes in sufficiently high yield and purity within a reasonable period of time. To overcome these difficulties we used the density perturbation technique with cationic colloidal silica beads.

Transformed human keratinocytes (line SV-K14 from B. Lane, London) were charged successively with the microbeads (a kind gift of Dr. B. Jacobson, Amherst) and polyacrylic acid prior to cell lysis by osmotic shock and mechanical shear. Owing to the high density of the microbeads, the coated plasma membranes could be easily separated from other cell organelles by centrifugation (30 min × 50,000 g) through a cushion of 60% (w/w) metrizamide.

The membrane preparation was characterized by Nomarski contrast microscopy, scanning and transmission electron microscopy, marker enzyme activities, DNA and protein content, one- and two-dimensional SDS polyacrylamide gel electrophoresis, and the beta-adrenergic receptor count. All the methods applied indicated a high degree of purification by this procedure. For example, the beta-adrenoceptors were in the range of (1.0 + 0.2) pmol/mg protein compared with (0.20 + 0.05) pmol/mg protein obtained for the 100,000 g particulate fraction from the same cell line without microbead treatment.

Experiments are in progress to adapt the technique for the preparation of plasma membranes from normal keratinocytes.

12. L-Serine Potentiates the Mitogenic Effects of Growth Factors on Keratinocytes in Culture. DAVID I. WILKINSON, Dermatology Dept., Stanford University School of Medicine, Stanford, CA.

L-serine improves the growth of cultured keratinocytes (KTs) when added to media such as Eagles MEM (Liu, 1978). This study describes effects of serine on KT growth in the presence of added growth factors.

Neonatal foreskin KTs were cultured primarily in Dulbecco MEM with fetal calf serum (FCS;9%), hydrocortisone (HC), cholera toxin and attenuated 3T3 cells as feeders. After passage using trypsin, KTs were cultured in Eagle MEM with FCS (4%), HC, and w/wo L-serine (0.8 mM). After 2 days, one or more of the following was added: insulin (4 μg/ml), epidermal growth factor (EGF; 10 ng/ml), bovine pituitary extract (BPE; 70 μg protein/ml; Boyce, 1984). Cell were cultured for 14 days and 2 dishes harvested daily. Growth curves in terms of DNA and protein per dish were assembled. Elsewhere, dishes of KTs were maintained for up to 4 days in medium with FCS, HC, serine (0.05 mM) and ^{3}H-serine (2 uCi/ml); at intervals, 2 dishes were rinsed, extracted with chloroform-methanol (2:1) and phospholipids separated by TLC, scraped off in bands and each band subjected to scintillation counting.

Without serine, KTs failed to proliferate and addition of growth factors had scarcely any effect. Serine stimulated growth even at 0.05 mM and strongly potentiated the effects of all 3 factors; maximum DNA levels (% of control) were: with EFG 742, insulin 390, BPE 325, serine alone 270. Maximum growth was observed with all 3 factors present (875%). ^{3}H-serine labelled phosphatidylserine initially but most ^{3}H was eventually found in phosphatidylethanolamine.

Serine may modulate agonist-receptor binding by altering plasma membrane phospholipid composition. In the absence of serine, growth factors have almost no effect.

CONCURRENT SESSION 2C: BULLOUS DISEASES

Federal

Hideoki Ogawa and John Stanley, Presiding

1. A Unique Intraepidermal Blistering Disease with Intercellular IgA Deposition. J. CLARK HUFF, KATHLEEN S. KUNKE, LAURA M. THOMASON, AND LOREN E. GOLITZ, Dept. of Dermatology, University of Colorado School of Medicine, Denver, CO.

Pemphigus vulgaris and pemphigus foliaceous, the only immune-mediated intraepidermal blistering diseases with intraepidermal deposits of immunoglobulin, are characterized by deposition of IgG autoantibodies between cells of the epithelium and often by circulating IgG autoantibodies which react with antigens present in the intercellular spaces of stratified squamous epithelium. In this report we wish to describe an unusual intraepidermal blistering disease with clinical, histologic, and immunologic features distinct from pemphigus.

In 1982 a 70 year old man developed a persistent moderately pruritic papulovesicular eruption with lesions over the skin of the trunk and proximal extremities but with no mucosal lesions. On multiple routine skin biopsies, there were intraepidermal blisters with minimal acantholysis, necrotic keratinocytes, neutrophils, and eosinophils. On direct immunofluorescence (IF) examination of multiple biopsies of involved and uninvolved skin, obtained over an 18 month period, strong IgA staining was present between epidermal cells above the basal cell layer. Indirect IF examination of the patient's serum on monkey esophagus demonstrated IgA staining of epithelial cell cytoplasm above the basal layer but no typical pemphigus pattern. The patient's serum also contained IgA antinuclear antibodies up to a titer of 1:500. No IgG autoantibodies were identified. The patient's disease responded poorly to topical steroids and oral prednisone up to 60 mg per day but cleared completely and has remained controlled on dapsone 50 mg every other day. The intraepidermal IgA deposits have persisted despite the drug-induced remission. The features in this case are not typical of pemphigus but represent a unique intraepidermal blistering disease with intercellular IgA deposits.

2. *In Vitro* Studies of Human Cutaneous Mast Cells from Patients with Mastocytosis. M. J. GLASS, M. B. CHAKER, L. SEELIG, AND M. D. THARP, Depts. of Dermatology and Cell Biology, UTHSCD, Dallas, TX.

Mastocytosis represents a spectrum of clinical disorders that results from an aberrant proliferation of tissue mast cells. The mechanisms of mediator release in this proliferating cell population has not been characterized. Therefore, we investigated the morphology and functional reactivity of skin mast cells from two patients with mastocytosis. Electron microscopy of lesional skin demonstrated a marked variation in mast cell size and granule number. Most of the cells had numerous atypical cytoplasmic villi; some were multinucleated. Following enzymatic digestion of lesional skin biopsies, the response of mastocytosis mast cells to several immunologic and nonimmunologic stimuli was investigated *in vitro*. 1.1×10^6 to 3.2×10^6 mast cells/gm of wet weight tisue (≥80% of total cells) were recovered with a mean viability of 85%. These cells were FITC-avidin and metachromatic stain positive and had an average histamine content of 2.8 (± 0.3 SEM) pg/cell. After passive sensitization with human IgE mastocytosis mast cells demonstrated a dose-related histamine response (maximal net release of 28.4%) following stimulation with murine monoclonal anti-human IgE. No histamine release occurred in unsensitized cells. Similar mast cell suspensions released histamine after stimulation with purified human C3a and calcium ionophore A23187. These studies indicate that atypical mast cells in patients with mastocytosis express functional receptors for IgE and C3a, and respond to a calcium ionophore stimulus. As with some patients with normal appearing mast cells, mastocytosis patients also are at potential risk for immunologically-mediated mast cell degranulation.

3. Cytoid Bodies and Human IgM Anti-keratin Filament Autoantibodies: A Pathogenetic Connection? GERHARD GRUBAUER, NIKOLAUS ROMANI, HEINZ KOFLER, PETER FRITSCH, AND HELMUT HINTNER, Dept. of Dermatology, University of Innsbruck, Innsbruck, Austria.

Cytoid bodies (CB), a frequent finding in diseased skin, derive from keratinocytes by apoptosis. They consist partly of keratin intermediate filaments (KIF) and bear Ig deposits—mostly IgM—thought to be due to non-specific trapping. Since IgM anti-KIF-autoantibodies occur in various diseases, we asked the question whether CB represent a regular and frequent feature in normal human skin and whether IgM anti-KIF-autoantibodies are—as a specific immune response—a constant finding in normal human sera. CB were visualized in 10 normal human skin specimens from various body sites by direct immunofluorescence with a FITC rabbit anti-human IgM conjugate and counted in both serial sections and dermal sheets, on one occasion also on EM. The KIF origin of CB was revealed by double staining with FITC rabbit anti-human IgM followed in sequence by a monoclonal mouse antibody against keratin and by a species specific Texas Red conjugated anti-mouse Ig. IgM anti-KIF-autoantibodies in 18 normal human sera and 2 sera from skin donors were demonstrated by immunoblot or measured semiquantitatively by an ELISA assay. The number of CB per mm^2 of normal human skin varied from 16 to 193 (20 on EM), most of them containing KIF proteins. IgM anti-KIF-autoantibodies were found in all test sera by immunoblot. In the ELISA system the mean extinction values (Em50) ranged from 0.15 to 0.339 as compared to an IgM myeloma reference serum in adequate dilutions (Em50 = 0.02). The presence of an enormous number of IgM-coated CB ($>1 \times 10^7/m^2$ skin) and the constant occurrence of IgM anti-KIF-autoantibodies in all normal human sera suggest that IgM deposits on CB represent a specific immune response and that, in general, the ubiquitous apoptotic cell death may provide a significant source of antigens for autoantibody production.

4. Immunoglobulins and Complement in Skin-limited Amyloids Can be Depleted by Chemical Pre-treatments. H. MUKAI, H. ETO, AND S. NISHIYAMA. Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Recently we have shown that amyloids in skin-limited amyloidoses are derived from epidermal keratinocytes (J. Invest. Dermatol. 1984, Am. J. Pathol. 1984). On the other hand, it has been reported that immunoglobulins (Ig) and complements (C) are consistently demonstrated in skin-limited amyloids and little is known concerning the relationship between amyloids and Ig or C. Present study was designed to clarify whether these Ig and C were parts of amyloids or due to non-specific absorption. Immunofluorescence studies were carried out on 19 patients with skin-limited amyloidoses; macular (10 cases), biphasic (4), lichenoid (2), familial (2) and skin tumor-associated (1). Without pre-treatment, amyloids in all the cases showed positive staining for Ig or C, particularly IgG, IgM and Clq. However, when we pre-treated these sections with 0.1M glycine buffer (pH 7.2) or 0.05% tween-20 solution for various periods, Ig and C in amyloid deposits turned out to be negative or only weakly positive. Even extensive washing with 0.1 M phosphate-buffered saline (pH 7.2) resulted in reduced positive staining. In amyloids lying close to dermal-epidermal junction, Ig and C had tendency to be depleted easily whereas those in deeper area showed tendency to remain weakly. Existance of amyloids before and after these treatments were confirmed by H&E, Thioflavin-T and Dylon stains. In addition, disulfide bonds in amyloids demonstrated by N-(7-dimehtylamino-4-methyl-3-coumarinyl)-maleimide (DACM) staining and the reactivities of amyloids against monoclonal antikeratin antibody EKH4 did not alter by these treatments.

These studies clearly indicated that Ig and C in these amyloids are result of non-specific absorption due to penetration of serum rather than parts of amyloids.

5. Characterization of KF-1 Monoclonal Antibody. BRUNO A. BERNARD, Dept. of Cell Biology, Centre International de Recherches Dermatologiques (C.I.R.D.), Valbonne, France.

The monoclonal antibody KF-1 identifies a non-collageneous component of the lamina densa of the basement membrane zone (BMZ) of skin (Breathnach et al., JID, 80:392, 1983). This component is absent in dystrophic forms of epidermolysis bullosa (Fine et al., JID, 82:35, 1984) and appears in the BMZ at the 16th week of human development, long after the appearance of laminin, collagen IV and Bullous Pemphigoid antigen (Fine et al., JID, 83:66, 1984). In the present work, we further characterize both the monoclonal antibody and the antigen.

By immunodiffusion technique, we show that KF-1 monoclonal antibody is an IgG_3 immunoglobulin. We also identify two squamous carcinoma cell lines—namely TR131 and TR146—which quantitatively express the antigen. Using the immunofluorescence technique, we show that the antigen is 1/ a cell surface antigen, 2/ sensitive to Triton X100 extraction, and 3/ concentrated in lamellar structures from which actin fibers are excluded.

Finally, by immunoblotting technique, we show that this monoclonal antibody identifies a 72kD polypeptide present in TR131 cell extract as well as in cultured human keratinocyte cell extract.

6. Expression of Pemphigoid Antigen by SV40-transformed Human Keratinocytes. NATSUKO OKADA, YUKIO KITANO, SACHIKO MIYAGAWA*, KUNIKI SAKAMOTO*, AND MARK L STEINBERG†, Dept. of Dermatology, Osaka University, Osaka, Japan, *Dept. of Dermatology, Nara Medical University, Nara, Japan, and †Dept. of Pathology, New York University, New York, NY.

Bullous pemphigoid (BP) antigen is a normal basement membrane zone antigen of epidermis and other squamous epithelia. Its biologic role is unknown; however it does appear to be synthesized in basal cells

and cultured keratinocytes. We have studied the expression of pemphigoid antigen by SV40-transformed human keratinocytes. Well characterized sera of 8 patients with BP (6), chronic localized pemphigoid (1), and drug induced lichen planus pemphigoides (1) were used as sources of autoantibodies. By indirect immunofluorescence, SV40-transformed keratinocytes in passage 80 through 85 expressed the antigen in coarsely granular fluorescence throughout the cytoplasm. Normal human serum demonstrated no specific staining on the cells. To characterize this antigen, 0.5% NP40 extracts of ^{14}C-amino acid labelled cells were immunoprecipitated using 8 different sera, subjected to SDS polyacrylamide gel electrophoresis and then fluorographed. All 8 sera precipitated a protein of approximate molecular weight 240 kd, while normal sera did not precipitate this protein. These results indicate that SV40-transformed human keratinocytes synthesize pemphigoid antigen, and that autoantibodies in the sera of patients with BP, chronic localized pemphigoid and drug induced lichen planus pemphigoides identify the same antigen of approximate molecular weight 240 kd.

7. Partial Purification and Characterization of the Major Bullous Pemphigoid (BP) Antigens as Intermediate Filament Associated Proteins. R. S. LABIB, D. F. MUTASIM, H. P. PATEL, G. J. ANHALT, AND L. A. DIAZ, Dept. of Dermatology, The Johns Hopkins University, Baltimore, MD.

Immunofluorescent and immunoelectron microscopic (IEM) investigations from our laboratories localized binding of BP autoantibodies to the hemidesmosomal plaque region. By immunoblotting of human epidemal extracts, we have identified 8 different antigens reactive with BP sera (n = 28). The two major antigens were 240 K and 180 K in molecular weight. Both antigens were undetectable by protein stains in SDS-PAGE of total epidermal extracts. The purpose of this study was to prepare an enriched preparation of these BP antigens.

We used sequential differential extraction of human heat separated epidermis in 5 solvents (JID 82:411, 1984) and immunoblotting as a detection system. The bulk of the BP antigens were recovered in the sodium dodecyl sufate (SDS) beta mercaptoethanol (BME) extract. They were not detectable in the cytoplasmic soluble, nonionic detergent soluble or salt dissociated protein fractions. This identified the antigens as components of the intermediate filament associated proteins which agrees with their hemidesmosomal plaque localization by IEM. The two major contaminants in the SDS-BME extract were the desmoplakins (250 K, 215 K) and keratins. Removal of the keratins from the preparation was desirable because of their abundance and the presence of autoantibodies against them in all human sera. This was achieved by gel filtration on a Biogel A-5m column equilibrated with a buffer containing 1% SDS and 2 mM dithiothreitol. The pooled active fractions represented a highly purified preparation of BP antigens suitable for immunological studies, further purification, and for more complete biochemical characterization.

8. Studies on Hemidesmosome-Cytoskeleton Interactions Using Bullous Pemphigoid Autoantibodies. D. F. MUTASIM, Y. TAKAHASHI, L. A. DIAZ, G. J. ANHALT, R. S. LABIB, AND H. P. PATEL, Dept. of Dermatology, The Johns Hopkins University, Baltimore, MD.

We have recently shown that bullous pemphigoid autoantibodies (BPab) bind specifically to hemidesmosomes (HD). Using this marker we studied the fate of the HD during trypsin dissociation of epidermal basal cells and the role of the cytoskeleton in this process.

Neonatal BALB/c mouse skin was incubated in 0.25% trypsin at 37°C for 20, 40 and 60 min. Samples of skin as well as the dissociated basal cells were processed for electron microscopy and/or immunoelectron microscopy using BPab. Similar studies were done while the tissue was exposed to different cytoskeleton disrupting agents.

After 40 min of trypsinization, intact epidermis separates from the dermis. Pits including portions of the dermal face of the basal cell membrane with HDs are seen. These pits ultimately pinch off and form vesicles. In the fully dissociated basal cells (60 min) intracellular vesicles having presumably both split desmosomes and HDs are seen close to the nucleus. Vesicles having HDs were detected by the specific deposition of BP immunoreactants on their surface and were limited to one pole of the basal cell. Perinuclear retraction of tonofilaments and ruffling of the plasma membrane were evident. HD internalization was inhibited by cold (4°C) as well as the presence of cytochalasin-D (1 μg/ml) and colchicine (100 μg/ml).

In summary, during trypsin dissociation of basal cells, HDs are internalized by a process which is dependent on the cytoskeleton. The specific binding of BPab to HDs may prove helpful in future biochemical and immunocytochemical studies of the HD.

9. The Linear Immunofluorescence Staining of the Cutaneous Basement Membrane Zone Produced by Pemphigoid Antibodies is the Result of Hemidesmosome Staining. D. F. MUTASIM, H. P. PATEL, R. S. LABIB, G. J. ANHALT, AND L. A. DIAZ, Dept. of Dermatology, The Johns Hopkins University, Baltimore, MD.

Hemidesmosomes (HD) are organelles scattered on the dermal pole of the basal cell membrane and, as shown in our previous studies, contain a major pool of bullous pemphigoid (BP) antigen(s). Moreover, BP antibodies (BPab) produce a linear staining of the cutaneous basement membrane zone (BMZ) by indirect immunofluorescence (IIF). This study demonstrates that this staining pattern results from the staining of individual HDs of the basal cell layer.

Cryosections of murine and human skin were obtained at vertical, oblique and horizontal planes to the epidermis and incubated with BPab. In addition, whole permeabilized epidermal sheets were incubated with BPab. Specific binding of BPab to these substrates was investigated by IIF. Epidermal sheets were mounted on glass slides with the basal cell layer facing up. In vertical sections of skin the BMZ staining was linear. This staining became wider and granular as the sections were made more oblique. In horizontal sections through the dermal pole of the basal cell layer as well as in whole epidermal sheets the staining was uniformly granular (carpet-like) and limited to individual basal cells. A nonfluorescent space separated individual basal cells. Normal human sera produced no staining. The granules detected within basal cells represent immunoreactants deposited in HDs as clearly shown by our immunoelectron microscopic examination of these specimens.

In summary, the BP linear IF staining results from having many of the discretely stained granules (HD) enclosed in a 4 μm thick vertical section of the lower pole of the basal cell layer of the skin.

10. Differential Staining of Acantholytic Cells in Pemphigus Vulgaris, Darier's Disease and Hailey-Hailey's Disease with Monoclonal Anti-desmosome Antibody HK1. HIKARU ETO, TOSHIO TAZAWA, MASAAKI ITO, MITSUHIRO MATSUMOTO, AND KEN HASHIMOTO, Depts. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan, Niigata University School of Medicine, Niigata, Japan, and Wayne State University School of Medicine, Detroit, MI.

Although desmosomes are thought to be important for cell-to-cell attachment in epithelial tissues, little is known concerning their rolls in acantholytic diseases. In the present study, monoclonal antibody HK1 which was developed by us and reacts with attachment plaques of desmosomes was used to study acantholytic cells in pemphigus vulgaris, Darier's disease and Hailey-Hailey's disease. In pemphigus acantholytic lesions, both acantholyzed and non-acantholyzed cells showed peripheral staining with HK1 antibody. This staining pattern was identical to that of keratinocytes in the normal epidermis. Immunoelectron microscopy confirmed that the staining was on desmosomes. Thus, desmosomes were relatively well preserved during cell detachment and, therefore, do not seem to be invovled in the process of acantholysis in pemphigus vulgaris.

In Darier's disease and Hailey-Hailey's disease, the majority of acantholyzed cells showed cytoplasmic staining in a diffuse pattern. This altered staining pattern was also observed in partially acantholyzed cells. Ultrastructurally, desmosomes were decreased in number or disappeared in these cells and HK1 antigen was diffused into cytoplasm, predominantly translocated on tonofilaments. Normal looking keratinocytes in non-acantholytic epidermis of both diseases did not show altered staining. Early changes of desmosomes during acantholysis demonstrated by HK1 antibody suggest involvement of desmosomes in the pathogenesis of Darier's disease and Hailey-Hailey's disease.

11. Monoclonal Antibody (ECS-1) with Pemphigus Antibody-like Properties. M. NEGI, L. A. GOLDSMITH, AND A. T. LANE, Dermatology Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Murine monoclonal antibodies to epidermal cell surface components were prepared by fusing NS-1 mouse myeloma cells with spleen cells of Balb/C mice immunized with cultured human foreskin keratinocytes. Hybrid cells secreting antibodies were screened by ELISA using epidermal extracts and indirect immunofluorescence techniques using frozen sections of neonatal foreskin as substrate.

One monoclonal antibody, (ECS-1), an IgM, showed selective staining on the cell surface of the nucleated layers in normal epidermis; reactivity with granular cell surfaces was stronger than that seen on the spinous and basal cell surfaces. Similar staining was seen in adult palm skin, mouse lip epidermis and colonies of cultured human keratinocytes. ECS-1 was detected in human fetal skin by 9 wks. of fetal life; the same age pemphigus antigen first appears.

Fluorescence flow cytometric analysis showed the antibody reacted with a cell surface molecule of the mouse epidermal cell line PAM: this cell surface molecule was trypsin sensitive. Immunoblot analysis showed that ECS-1 reacted with a 36Kd protein extracted from neonatal foreskin epidermal cells and cultured human keratinocytes. The protein required SDS and mercaptoethanol for solubilization. The protein identified by ECS-1 is similar in molecular weight to that previously described for a pemphigus antigen (J Clin Invest *73*:1113, 1984).

The distribution, age of appearance in fetal life and molecular weight of its antigen suggests that ECS-1 may be a pemphigus-like monoclonal antibody. Studies to induce pemphigus lesions in neonatal mouse skin and epidermal tissue and organ cultures using this monoclonal antibody are in progress.

12. Specificity and Pathogenicity of Animal Pemphigus Antibodies. J. G. HUANG, E. H. BEUTNER, AND R. M. LEWIS, Dept. of Microbiology, University at Buffalo, SUNY, Buffalo, NY, and Dept. of Pathology, NYS College of Veterinary Medicine, Ithaca, NY.

Pemphigus has been described in dogs, cats, horses and recently in goats, based on clinical, histologic, and immunohistologic findings. However, characteristics of circulating antibodies to epithelial intercellular antigens (ICAb's) have not been analyzed serologically to date.

Canine and equine pemphigus sera (CPS, EPS) were analyzed by indirect immunofluorescence and a mixed competitive inhibition assay to characterize their specificity, and standard organ culture methods to determine their pathogenicity. We found that the ICAb's in CPS and EPS demonstrated tissue specificity for stratified squamous epithelium (SSE), and exhibited limited, variable species specificity. That is, animal ICAb's cross-reacted on SSE of many, but not all, species tested. Both CPS and EPS competitively inhibited the binding of human pemphigus antibodies to SSE; the converse was also true. The degree of inhibition varied with the species source of SSE. ICAb's from CPS bound in organ cultures of canine esophagus. Acantholytic changes occurred in these cultures after 48 hrs., but did not occur in parallel cultures incubated with normal canine serum.

Despite the less consistent occurrence of circulating ICAb's in animal pemphigus than in human disease, animal ICAb's when present, exhibit similar characteristics of binding specificity and pathogenicity to human pemphigus antibodies.

CONCURRENT SESSION 2D: CLINICAL RESEARCH I

South American

Brian Jegasothy and Muneo Okido, Presiding

1. Characterization of Immunoreactive cAMP-Phosphodiesterase in Atopic and Histamine-Treated Normal Monocytes. S. C. CHAN, W. J. THOMPSON, AND J. M. HANIFIN, Oregon Health Sciences University, Portland, OR, and University of Southern Alabama, Mobile, AL.

The highly active cAMP-phosphodiesterase (PDE) in monocytes of patients with atopic dermatitis (AD) and the histamine-sensitive normal monocyte-PDE react with a specific antibody against 60,000 MW PDE. We have used this antibody to determine whether the increased PDE activity is due to activation or to induction of enzyme.

Monocytes were isolated from AD and normal blood on Hypaque-Ficoll and Percoll gradients. Sucrose gradient-fractionated monocyte PDE's were applied to SDS gel electrophoresis. The resulting gels were electro-blotted and 60K PDE was identified by ^{125}I-antibody (sheep anti-dog kidney PDE) autoradiography, then quantitated by densitometry and expressed as immunoreactivity units (IR). PDE was measured by two-step hydrolysis of ^{3}H-cAMP (1 unit = 1 pmol/min/mg protein).

IR in unfractionated AD monocyte homogenates was 1213 ± 115 units/mg compared with 606 ± 286 units in normal preparations. Respective PDE activities were 69.8 ± 15.8 and 11.2 ± 4.0 units. In the 3.6S and 5.6S sucrose gradient fractions, AD monocyte PDE IR's were 50% and 33% greater than normal, respectively. These increases were inadequate to account for the 3-fold and 2-fold elevations of PDE enzyme activity in these respective fractions. Histamine (1 μM at 37°C for 1 hour) stimulated 4-fold increased PDE activity in normal monocytes with no corresponding increase in IR. AD monocytes have quantitatively increased PDE but, in addition, may have increased activation of enzyme by mediators such as histamine. Histamine activation of PDE with consequently lower cAMP may result in permissive functional regulatory responses in mast cells and other inflammatory components.

2. Eosinophil Granule Major Basic Protein Deposition in Atopic Dermatitis. K. M. LEIFERMAN, S. J. ACKERMAN, H. A. SAMPSON, H. S. HAUGEN, AND G. J. GLEICH, Depts. of Dermatology and Immunology, Mayo Clinic, Rochester, MN, and Dept. of Pediatrics, Duke University Medical Center, Durham, NC.

Although eosinophils are not prominent in the infiltrate in lesions from patients with atopic dermatitis, peripheral eosinophilia is seen. The eosinophil granule major basic protein (MBP) constituting the granule core is toxic to helminths and mammalian cells, and induces mediator cell activation. To determine whether eosinophil degranulation occurs in lesions of atopic dermatitis and other diseases with lichenification, we performed an indirect immunofluorescence assay on sections of formalin-fixed, paraffin-embedded tissue, utilizing affinity chromatography purified antibody to MBP. Twenty biopsies from patients with atopic dermatitis, 8 from patients with chronic untreated onchocerciasis, 2 each from patients with prurigo nodularis, neurodermatitis and lichen simplex chronicus were studied. Biopsies from 3 patients with acute contact dermatitis were also studied. Twenty biopsies from 18 patients with atopic dermatitis all showed evidence of MBP deposition outside of eosinophils. The staining was mainly fibrillar, located in the upper half of the dermis, and resembled the distribution of elastic fibers. It was nearly identical to that seen in 7 of 8 chronic lesions from untreated onchocerciasis patients. Similar but less extensive fibrillar fluorescence was seen in lesions from patients with prurigo nodularis and neurodermatitis. No extracellular fluorescence was seen in lichen simplex chronicus lesions or acute contact dermatitis. These results suggest that eosinophils commonly degranulate in the dermis and point to a role of eosinophil granule proteins in the pathogenesis of atopic dermatitis and other disorders with lichenification.

3. Monoclonal Antibody-Defined Human Melanosomal Properties Common to Divergent Oncogenic Differentiation of Pigment Cells and Their Cell Membrane. KAZUHITO HAYASHIBE, YUTAKA MISHIMA, MICHIO KAWAI, AND MASAMITSU ICHIHASHI, Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan.

Interrelationship of melanoma-associated antigens and melanosomal antigenic properties has been investigated in relation to divergent oncogenic differentiation of melanosome synthesizing cells. BALB/c mice were immunized with purified melanosomal fraction from human SK-MEL-12 cells and subsequently spleen cells were fused with mouse myeloma cell line P3/X63.Ag8.U1. The specificity of the established monoclonal antibody, A4F11 against each subcellular fractions and cell surface were checked by RIA, indirect immunofluorescence and immuno-electron microscopy of cultured SK-MEL-12 cells using saponin cell permeation method.

A4F11 has been found to bind selectively with melanosomal and small granule fractions of SK-MEL-12 cells. Analysis by immunofluorescence has detected the cytoplasmic reactivity in cultured SK-MEL-12 cells and malignant blue nevus cells. Electron microscopy using the saponin method has revealed that AF4F11 is localized on the melanosomes and GERLs as well as on the cell surface. The affinity of A4F11 to the cell surface has been confirmed by indirect ^{125}I-labelled binding assay. This anti-melanosomal antibody has also been found to react with normal junctional melanocytes, compound nevus cells and nodular melanoma cells with distinct cytoplasmic granular reactivity on frozen sections.

The above evidence would indicate the presence of antigenic determinant common to intracellular melanogenic compartments and on the cell surface of human pigment cells, regardless to their oncogenic differentiation status.

It is further suggested that other cell types with unique protein synthesis may also possess specific antigenic determinants on their cell membranes which are unique to their characteristic organelles.

4. Factor VIII-Related Antigen (VIIIR-Ag) in Kaposi's Sarcoma. N. S. PENNEYS, RECIA KOTT-BLUMENKRANZ, AND FRANCISCO CIVANTOS, Depts. of Dermatology and Pathology, University of Miami School of Medicine, Miami, FL.

Kaposi's sarcoma is a spindle cell neoplasm of vascular origin. The endothelial derivation of this tumor is supported by positive immunohistochemical identification of blood group antigens and VIIIR-Ag within tumor cells. VIIIR-Ag is a substance that is synthesized within endothelial cells and is an established immunohistochemical marker for these cells. In this study, we have examined the plasma of patients with cutaneous Kaposi's sarcoma for alterations in levels of VIIIR-Ag.

Blood was collected from 10 adult male patients who did not have acquired immune deficiency syndrome but who had biopsy proven cutaneous Kaposi's sarcoma. VIIIR-Ag levels were determined on plasma samples by the method of rocket electrophoresis in a research hematology laboratory. VIIIR-Ag levels were found to be elevated in 9 of 10 patients. The mean value was 298 + 132% with normal values in this laboratory ranging from 70% to 150%. Patients with more extensive disease tended to have higher levels of VIIIR-Ag in their plasma.

The results of this study suggest a significant association between cutaneous Kaposi's sarcoma and the presence of elevated plasma levels of VIIIR-Ag. VIIIR-Ag levels were increased even though cutaneous involvement was minimal in many of these cases. This association may provide a useful diagnostic and prognostic marker for cutaneous and internal involvement by this neoplasm in non-acquired immune deficiency syndrome and acquired immune deficiency syndrome patients.

5. A New Monoclonal Antibody Which Identifies Endothelial Cells (EC). S. MATSUO, N. PENNEYS, M. NADJI, J. ZIEGELS-WEISSMAN, AND K. ADACHI, Depts. of Dermatology and Pathology, University of Miami School of Medicine, Miami, FL, and Dept. of Dermatology, Asahikawa Medical College, Hokkaido, Japan.

The demonstration of antigen characteristic of a specific cell type within a tumor cell supports the concept that the tumor cell is derived from that parent cell. We are generating an array of monoclonal antibodies to tissue antigens that are stable to formalin fixation and paraffin embedding; such antigens, when present in a neoplasm, would be of help in identification and classification of the neoplasm.

Standard mouse-mouse hybridoma methodology was used after immunization with homogenate of human thoracic duct and associated connective tissue. The supernatants of viable colonies were assayed for antibody by an unlabelled antibody peroxidase-antiperoxidase method using sections of formalin-fixed, paraffin embedded thoracic duct as substrate. An antibody was identified to an antigen of molecular weight 68 Kd present in lymphatic cells, EC, and EC-associated basement membrane. Six cases of Kaposi's sarcoma, 3, of angiosarcoma, 15 of leucocytoclastic vasculitis, and a variety of controls were examined at various dilutions of the primary antibody and using appropriate controls in each experiment.

The monoclonal antibody identified an intracellular antigen in all cases of Kaposi's sarcoma and angiosarcoma. Non-EC-derived spindle cell lesions did not contain this antigen. EC could be identified in vessels damaged by leucocytoclastic inflammatory processes. Little background staining was observed. This antibody will be of diagnostic use in identifying EC-derived neoplasms as well as in studying the effects of inflammation on EC and their basement membrane.

6. Purification and Characterization of a Major Extracellular Proteinase from *Trichophyton rubrum (T. rubrum).* MASAKAZU ASAHI, ROBERT LINDQUIST, KIMIE FUKUYAMA, GERARD APODACA, WILIAM L. EPSTEIN, AND JAMES H. MCKERROW, Depts. of Dermatology and Pathology, University of California, San Francisco, and Dept. of Chemistry, San Francisco State University, San Francisco, CA.

T. rubrum, the most common dermatophyte of man, is known to release proteolytic enzymes which digest keratin. This paper reports purification and characterization of the major proteinase from a strain of *T. rubrum*. Proteins in the culture filtrate were precipitated by adding ammonium sulfate (70% saturation) and applied on a Sephadex G-100 column. Proteolytic activity was further purified from a broad G-100 peak using a Mono-P chromatofocusing column eluted with a pH 8-5 gradient. Homogeneity was judged by silver staining on SDS polyacrylamide gel electrophoresis. The major proteinase has a pI of 7.8 and a subunit M_r of 42,000, but appears to exist in the "native" state as a 92,000 M_r dimer detected by gel electrophoresis without dithiothreitol. The proteinase degraded Azocoll, ^{3}H-elastin, ^{14}C-keratin, casein and gelatin with an optimal pH of 8. Kinetic studies also showed that tetrapeptides containing aromatic or hydrophobic residues at P1 were the best substrates. A k_{cat}/K_m of 27,028 $M^{-1} \cdot sec^{-1}$ was calculated for the peptide SuAlaAlaProPhe-pNA. The enzyme was inhibited by chlorylmethylketone tetrapeptides with hydrophobic residues at P1 as well as other inhibitors of serine proteinases. We suggest that the extracellular proteinase probably plays a central role in the metabolism and pathogenesis of *T. rubrum* infection.

7. The Biological Role of Keratinolytic Proteinase (KPase) and Its Inhibitor on the Growth of *Candida albicans.* R. TSUBOI, Y. KURITA, K. MATSUDA, M. NEGI, AND H. OGAWA, Dept. of Dermatology, Juntendo University School of Medicine, Tokyo, Japan.

In order for *C. albicans* to be parasitic on the stratum corneum, it would be expected to digest insoluble keratin proteins and acquire nutritive substances by releasing some form of proteolytic enzyme. With the electron microscope we observed morphologically that fungi were located within the region of the stratum corneum and were found to have digested the marginal band of the horny cell, and to have invaded the cytoplasm. Subsequently we found *C. albicans* produced a keratinolytic proteinase (KPase) when cultivated in liquid medium containing human stratum corneum (HSC) as the nitrogen source, but was unable to do so when cultivated in sabouraud dextrose broth, a medium frequently used in laboratories. Purified KPase from the culture supernatants belonged to the carboxyl proteinases group, had a molecular weight of 42.000 and an optimum pH 4.0. The activity of the enzyme was inhibited strongly with the addition of pepstatin. KPase could digest and be inducted from the membranous fraction, which is the most resistant structure of the horny cell. Suspecting that the enzyme may play an important role when *C. albicans* invades the epidermis, we investigated the influence of culture medium pH and various kinds of protease inhibitors on the growth of *C. albicans*. A rapid growth of *C. albicans* was observed with weakly acidic media, particularly at pH 4.0, while even at a neutral pH condition, *C. albicans* could change the surrounding pH into a weakly acidic pH and produced KPase. From amongst various kinds of protease inhibitors, pepstatin, a carboxyl protease inhibitor, most strongly inhibited the growth of *C. albicans* dependent upon its concentration and had a stronger antifungal effect than the well-known antifungal agent, clotrimazole. These morphological and biochemical results suggest that KPase plays an important role when *C. albicans* invades the epidermis and that pepstatin has a possibility of being used as a new type of antifungal agent.

8. Immunopathologic and Neutrophil Function Monitoring of Experimental Therapy in Behcet's Syndrome. JOSEPH L. JORIZZO, FRANK C. SCHMALSTEIG, ALVIN R. SOLOMON, JERRY C. DANIELS, AND TITO CAVALLO, Depts. of Dermatology, Pediatrics, Internal Medicine, and Pathology, University of Texas Medical Branch, Galveston, TX.

Ten patients with rigidly defined, clinically active, Behcet's syndrome have been evaluated over a 3 year period. Pathergy testing, the induction of a sterile pustular vasculitic lesion 24 hours after dermal injury by needle prick or intradermal injection, was assessed using intradermal injection of 0.05 ml of a 1.0 mg/ml solution of histamine phosphate. Lesions were evaluated by direct immunofluorescence microscopy (4 hours) and routine microscopy (24 hours) with appropriate controls. Neutrophril migration was assessed by our published subagarose method using patient or control serum in the agar. Neutrophil surface glycoproteins (Mol, Macl, OKM1, LFA1, p150.95) which may be involved in neutrophil adherence were evaluated qualitatively by tritium labeling of the cell surfaces of intact neutrophils. All patients showed evidence of immunoreactant deposition in dermal blood vessel (4 hours) and/or evidence of Sweet's-like or leukocytoclastic vasculitis (24 hours) after histamine injection during active disease. All patients showed evidence of serum enhancement of neutrophil migration. Neutrophil surface glycoproteins were not different from control neutrophils in the 5 patients tested. Oral thalidomide 200 mg nightly was given to 5 consenting adults as per our FDA issued IND. Clinical improvement was dramatic and immunohistopathologic evaluation of histamine induced pathergy testing became negative in 4/5 patients. Unlike colchicine therapy (which in our previous study abolished serum enhancement of neutrophil migration in 4 patients) thalidomide therapy had no effect on neutrophil migration or surface glycoproteins in this small patient group.

9. Immunohistochemical Analysis of the Mechanisms Underlying Spontaneous Regression Phenomenon of Numerous Flat Warts. H. TAGAMI, S. AIBA, AND M. ROKUGO, Dept. of Dermatology, Tohoku University School of Medicine, Sendai, Japan.

Numerous flat warts, viral tumors induced by human papillomavirus (HPV) types 3, 10 or 28, regress spontaneously within one month after

sudden occurrence of inflammation in all the warts, leaving permanent immunity to flat warts in the host. Thus, the study of this phenomenon provides a valuable direct information on tumor immunity in man. Histologically such inflammed flat warts show a dense mononuclear cell infiltration sharply confined to wart tissue. There is, however, little data on the characteristics of lymphoid cells as well as the role of HPV antigen in this immune-mediated tumor rejection phenomenon in man.

We studied the presence of HPV antigen by peroxidase-antiperoxidase test using genus-specific antiserum prepared from bovine papillomavirus. Positive reactivity for HPV was disclosed in none of 31 inflamed flat warts in contrast to 63% of 19 ordinary flat warts. The T cell phenotype assessment in 5 inflamed flat warts using monoclonal antibodies with immunoperoxidase technique showed that the lymphoid cells invading the epidermis consisted mostly of suppressor/cytotoxic subsets, whereas helper/inducer subsets predominated the infiltrating cells in the dermis. Natural killer cells were absent. HLA-DR antigens were demonstrated not only on Langerhans cells and lymphoid cells but also on the surface of the keratinocytes.

These data indicate that, although flat wart is a viral tumor, the major immune mechanism underlying its spontaneous regression phenomenon is not an immune reaction against HPV antigen present in the nuclei of upper epidermal cells but a T cell-mediated attack against tumor cells.

10. Killed *T. mentagrophytes* Spores Differ from Hyphae in Their Ability to Induce Cutaneous Reactions and Activate Complement. R. E. REECE, A. H. ZUCKER, AND A. D. HERNANDEZ, VA Medical Center and University of Tennessee Center for the Health Sciences, Memphis, TN.

The clinical manifestation of *T. mentagrophytes* infecton differs depending on whether stratum corneum or hair is infected. Stratum corneum infections are characterized by erythema and scale while hair infections are characterized by pustules. Hyphae predominante in stratum corneum infections while spores predominante in hair infections. To determine whether the predominant form of the fungus was responsible for inducing the clinical manifestation of stratum corneum and hair infection, we inoculated the skin of guinea pigs with killed *T. mentagrophytes* spores or hyphae. Only guinea pigs previously infected with *T. mentagrophytes* developed cutaneous reactions when incoluated with killed spores or hyphae. Spores elicited a pustular reaction in 4 of 6 guinea pigs while 3 of 6 guinea pigs inoculated with hyphae developed scale and erythema. To determine whether the spore form of *T. mentagrophytes* activated more C′ than hyphae on a weight basis, we measured the ability of spores and hyphae to activate C′. Equal weights of spores or hyphae were incubated with serum and the activation of C_3 and C_5 determined using immunoelectrophoresis and a radioimmunoassay, respectively. Both spores and hyphae activated C_3 and C_5. Activation occurred through both the classical and alternate pathways. Serum incubated with spores generated more C_{5_a} than those incubated with hyphae. These results show that spores or hyphae of killed *T. mentagrophytes* elicit two distinct clincial manifestations in guinea pigs which resemble those observed in man. The data suggests that the predominant form of the organism determines whether pustules or scales are elicited, and that this may be mediated through C′ activation.

11. Epidermal Growth Factor (EGF) Receptors in Smooth Muscle Cells *In Vitro*. C. ARZUBIAGA, C. M. STOSCHECK, AND L. E. KING, JR., Div. of Dermatology, Vanderbilt University and V.A. Medical Centers, Nashville, TN.

In mammalian skin, 1251-EGF binds to epithelial and nonepithelial cells such as smooth muscle (SM) cells found in or around hair follicles, sweat glands and blood vessels (Nanney et al, J. Invest. Dermatol 83: 989, 1984). To characterize the EGF receptors in SM, cells from the tunica media of rat thoracic aorta were obtained, grown *in vitro* in 1% fetal calf serum, (FCS) with or without added 1251-EGF. Cell membrane of these rat SM cells was also prepared, incubated with EGF and [Y-32P]ATP, and analyzed by autoradiography of SDS polyacrylamide gel electrophoresis (PAGE). EGF (10 ng/ml) stimulated the growth of these rat SM cells by 38% in 96h, as compared to SM cells grown in FCS alone. 1251-EGF binding to rat SM cells was saturated at 20 ng/ml EGF at 30 min and at 10 ng/ml at 4h at 37 C. Maximal 1251-EGF binding occurred at 30 min with a subsequent decline in cell bound 1251 in the ensuing 4h. Chloroquine (50 ng/ml) prevented the decline in SM bound 1251 when added to the medium. PMA (4B-phorbol-12B-myristate, 13a-acetate) inhibited 1251-EGF binding to these rat SM cells. Saturating concentrations of PMA (3 ng/ml) only inhibited 70% when 0.25 mg/ml) EGF was added to the SM cells. When analyzed by SDS PAGE the autoradiographs showed a major 170-kDa endogenous phosphorylated protein that was the only substate whose phosphorylation was EGF dependent. Under the conditions used to prepare the SM membranes (EGTA, protease inhibitors) the EGF receptor-kinase is expected to be a 170-kDa protein and not have a major degradation form of 150-kDa.

Conclusions: Rat SM cells *in vitro* have functional and specific EGF receptors that are saturable, down-regulated in a chloroquine inhibitable process and inhibited by the phorbol ester, PMA. EGF stimulated the growth of these rat SM cells and induced phosphorylation of a single 170-kDa membrane protein, presumably the EGF receptor-kinase itself.

12. In Vitro Studies on Pathomechanism of Fixed Drug Eruption. SHIGERU SAKAKIBARA, TOKIO NUMATA, AND NOBUYUKI MIZUNO, Dept. of Dermatology, Nagoya City University School of Medicine, Nagoya, Japan.

The characteristics of the fixed drug eruption are fixed site of lesion, specificity of drug, immediate type reaction, severe epidermal damage comparing with dermal one, and occasional spontaneous cure. A complete pathomechanism to explain these facts fully has not been proposed to date. To clarify the pathomechanism, in vitro studies were performed. Keratinocyte migrates of skin explant obtained from a patient with a fixed drug eruption due to sulindac (D), were challenged by several kinds of test samples, and then their morphological changes were observed. By addition of a patient's serum obtained 2 hours after ingestion of D [P(+)], migrated keratinocyte from the involved skin shrank, then detached from the Petri dish and disappeared. The same results were obtained by challenge of a heat inactivated P(+) and a patient's serum without D ingestion [P(−)] mixed with D in vitro. No significant changes were observed with D alone or a serum from a normal person obtained after D ingestion. The positive reaction was not observed with the uninvolved skin explant challenged by P(+), P(−), and P(−) mixed with D. Preincubation of the involved skin explant with anti-human lymphocyte antibody did not influence these reactions. These results indicate that the patient's lesional epidermis and serum factor and D are essential to produce the epidermal damage, and that complement system and lymphocyte may not be involved in this reaction. A $(NH_4)_2SO_4$ 0–50% Ppt. fraction of the patient's serum gave a strong reaction, but its supernatant gave a weak one. Addition of anti-IgA antibody to a mixture of P(−) and D, suppressed the reaction, but addition of either anti-IgG or anti-IgM antibody did not. So the serum factor may be IgA.

10:30 AM–12:30 PM Second Floor

POSTER SESSION I

Authors at Posters for Discussion

1. Detection of Anti-Gliadin Antibodies in Bullous Diseases and Their Recognition of Same Antigenic Polypeptides. VIJAY KUMAR, NARESH JAIN, AND ERNST H. BEUTNER, Depts. of Microbiology and Dermatology, University at Buffalo, SUNY, Buffalo, NY.

Most of the patients with dermatitis herpetiformis (DH), a pruritic vesicular skin disorder, have jejunal villous atrophy identical to that found in celiac disease. The gluten sensitive enteropathy is, however, less severe and both the gut and skin lesions improve on gluten free diet. Most DH patients have circulating antigliadin antibodies (AGA). However, there are conflicting reports as to the disease specificity of AGA. We studied the disease specificity of AGA in various bullous diseases to determine if they react with same gliadin polypeptides.

AGA were detected in the sera of 23 DH patients, 16 pemphigoid, 9 pemphigus and 57 normal controls both by indirect IF on monkey esophagus and by ELISA. Reactivities of AGA for various gliadin polypeptides were determined by immunoblot. AGA were positive in 20 of 23 DH, 12 of 16 pemphigoid, 7 of 9 pemphigus patients and 16 of 57 normal controls by ELISA. AGA from patients with various bullous diseases recognized the same five major polypeptides as in DH by the immunoblot method. The incidence was somewhat lower by the indirect IF method. We thus conclude that neither the presence of AGA nor of antibodies to specific gliadin peptides can be regarded as a disease specific marker of DH.

2. Altered Epidermal Cell Viability Induced by Pemphigus Antibody and Complement. S. KAWANA, W. D. GEOGHEGAN, AND

R. E. JORDON, Depts. of Dermatology, The University of Texas Health Science Center, Houston, TX, and Kitasato University School of Medicine, Sagamihara, Japan.

We have previously shown that complement (C) is fixed by pemphigus IgG to the surface of epidermal cells and enhances pemphigus IgG mediated detachment of monolayers of cultured epidermal cells. The present study extends these findings by investigating the effects of pemphigus IgG and C upon cell viability using trypan blue exclusion, ethidium bromide (EB) staining and fluorescein diacetate (FDA) conversion by living cells. Forty eight hour cultivated epidermal monolayers of neonatal BALB/c mice were incubated in media containing 1 mg/ml purified pemphigus IgG for 48 hours either in the presence or absence of C. Adherent and detached cells were examined by both phase and fluorescence microscopy.

Results from trypan blue exclusion showed that pemphigus IgG plus C produced some decrease in cell viability when compared to pemphigus IgG without C (adherent cells 68% versus 85% viable, detached cells 18.9% versus 36.6% viable, respectively). However, when FDA/EB comparisons were made, the differences were significant. In the absence of C or Clq or after 56°C for 30 min., the presence of pemphigus IgG resulted in an average of 54% FDA+ adherent cells and 13% EB+ detached cells. In the presence of C or plasminogen depleted C, there were 25% FDA+ adherent cells and 41% EB+ detached cells. Normal IgG with or without C yielded values of 61% FDA+ adherent and 9% EB+ detached. *These studies suggest that pemphigus antibody in the* presence of C alters cell viability and supports the contention that C activation contributes to acantholysis.

3. Regional Variation in the Expression of Pemphigus Vulgaris Antigen in Human Skin. L. SISON-FONACIER, M. SABOLINSKI, AND J-C. BYSTRYN, Dept. of Dermatology, NYU School of Medicine, New York, NY.

To examine whether pemphigus vulgaris (PV) antigen is expressed uniformly over the epidermis, we measured its concentration on different areas on the body. Three sera with high titers (640–1280) of PV antibodies, and as control three sera with bullous pemphigold (BP) antibodies, were reacted with 46 specimens of normal skin obtained from different individuals. All skin specimens were immediately frozen in liquid nitrogen and tested within three days. We found that the expression of PV antigen, determined from the highest dilution of PV antibodies giving a positive reaction by indirect immunofluorescence on a skin specimen, was greatest on scalp, face, neck, buccal mucosa, and axilla. The average concentration of PV antigen in 20 skin specimens tested from these areas was 320 (range 40–1280). PV antigen concentration was less on skin from lower back and groin. The average concentration in 6 specimens tested from these areas was 40 (range 0-320). There was an intermediate expression of PV antigen in the other areas tested (chest, abdomen, upper back, and legs). As previously shown, there was also regional variations in the expression of BP antigens measured concurrently in the same specimens of skin. However, the pattern of distribution of PV and BP antigens was strikingly different. Some areas strongly expressing PV antigens (scalp, face, neck) expressed little BP antigen; and conversely, BP antigen was strongly expressed in groin and low back which expressed little PV antigens.

These results indicate that there are regional variations in the expression of pemphigus vulgaris antigen and that the distribution of this antigen differs from that of bullous pemphigoid antigen.

4. Cytochemical and Biochemical Localization of Acid and Neutral Lipase Activity in Epidermis. GOPINATHAN K. MENON, STEPHEN GRAYSON, AND PETER M. ELIAS, Dermatology Service, VAMC, and Dept. of Dermatology, University of California School of Medicine, San Francisco, CA.

Despite a wealth of new information on epidermal lipids and their role in permeability barrier function and desquamation, little is known about the location of the enzymes that regulate their catabolism. In this study we have localized acid and neutral lipase in the outer epidermis simultaneously by cytochemical and cell fractionation techniques. Aldehyde-fixed tissues (100 μm slices) incubated in Tween 85 or triolein plus taurocholate/calcium chloride-containing buffer, pH 7.2 or 4.5, were then exposed to lead to form an insoluble "soap", and processed for electron microscopy. Simultaneously, cell homogenates and isolated lamellar body fractions were incubated with methylumbilliferone oleate (MUO) under similar conditions, with released, free MU servings as an index of lipase activity. On electron microscopy, both acid and neutral lipase were localized solely to intercellular domains in the stratum corneum (SC), with neutral lipase interspersed among intercellular lamellations, and acid lipase adjacent to the plasma membrane, but concentrated in the outer layers of the SC. In the stratum granulosum lipases were found, both ultrastructurally and biochemically in lamellar bodies, but neutral lipase also was localized in the perinuclear cistern mitochondria. In summary, these studies 1) by demonstrating lipid-catabolic enzymes in the stratum corneum, lend further support to the two-compartment model of the SC; 2) provide new information about the location of lipid-catabolic enzymes in differentiating epidermis; and 3) provide new insights about how lipids are processed during permeability barrier formation and desquamation.

5. De Novo Synthesis of Lysozyme by Human Epidermal Cells. VIRGINIA L. CHEN, DENNIS S. FRANCE, AND GIORGIO MARTINELLI, Dept. of Dermatology, Mount Sinai School of Medicine, New York, NY.

Lysozyme, a low molecular weight (14,500) cationic protein, is a well known bacteriolytic agent that hydrolyzes mucopeptides of bacterial cell walls. Although the presence of lysozyme has been reported in human skin, whether or not epidermal cells can directly synthesise this protein has not been determined.

Fresh normal human skin obtained at surgery was keratomed and incubated in 0.5% trypsin at 4°C for 24 hours and the epidermis removed from dermis. Epidermal cells were cultured in serum-free medium RPMI 1640 lacking leucine supplemented with antibiotics and containing 500 uC_i (^{3}H)-leucine in 5% CO_2 atmosphere at 37°C for 48 hours. Sonicated epidermal cell preparations were dialyzed to remove free (^{3}H)-leucine and concentrated. These preparations were electrophoresed on SDS-PAGE gel and analyzed by Western blot technique. Proteins transferred to nitrocellulose paper were immunostained with rabbit anti-human lysozyme antibody using an indirect immunoperoxidase method. A parallel gel was dried and processed by autoradiography. Coomassie blue staining revealed a major band at molecular weight 14,500 coincident with both an immunoreactive band and a major radioactive band. Bacteriolytic activity was demonstrated in fractions containing radioactive and immunoreactive proteins from partially purified human epidermal lysozyme.

These findings suggest that a bacteriolytic, immunoreactive lysozyme is one of the major proteins newly synthesized by human epidermal cells in short-term culture. This observation provides new evidence that epidermal cells may have the ability to protect the skin from bacterial infection by providing its own antibacterial agent.

6. Intracellular Viscosity Changes in Guinea Pig Keratinocytes Measured by Fluorescence Polarization. HIROSHI HACHISUKA, HIROFUMI NOMURA, YOICHIRO SASAI, KAZUHIDE SHIOTSUKI, AND M. MITSUO YOKOYAMA, Depts. of Dermatology and Immunology, Kurume University School of Medicine, Kurume, Japan.

During the process of epidermal cell keratinization, keratinocytes undergo dramatic changes, including synthesizing of prekeratin and keratin, formation of tonofilament and keratohyalin granules, and increasing their cell size. In this study, we used the fluorescence polarization technique to measure the intracellular viscosity of guinea pig keratinocytes. Guinea pig epidermal sheet was obtained with EDTA-PBS treatment and was incubated in 0.25% Trypsin-PBS for 20 min at 25°C. Epidermal cells were mixed with Percoll-Hanks solution and centrifuged at 20,000G for 30 min at 25°C and fractionated into high (HDF), intermediate (IDF) and low (LDF) density fractions. Morphological observation and cytoflourometric analysis of DNA content of these fractionated epidermal cells showed that HDF, IDF, and LDF were basal cell, squamous cell and granular cell-rich fractions, respectively. Two-tenth ml of the epidermal cell suspensions were incubated with 3 ml of FDA solution (fluorescein diacetate; 0.25 μg/ml, pH 7.4). Intracellular fluorescence polarization of each fraction was determined with a polarization spectrofluorometer (Hitachi MPF4) using excitation wavelength at 470 nm and an emission wavelength at 510 nm and P (polarization)-value was calculated. P-value of HDF, IDF and LDF was indicated as 0.195, 0.172 and 0.154, respectively. Intracellular viscosity of keratinocytes was higher in basal cells and lower in granular cells. Our result indicates that intracellular viscosity of guinea pig keratinocytes is descreased during keratinocytes differentiation.

7. Linoleate-rich Polar Lipids of Epidermis: Structure Determination by Proton Magnetic Resonance. WILLIAM ABRAHAM, PHILIP W. WERTZ, AND DONALD T. DOWNING, The Marshall Der-

matology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

The acylceramides (AC) and acylglucosylceramides (AGC) in mammalian epidermis are of particular interest because of their probable role in the barrier to water diffusion. We showed previously that in the AC, linoleic acid is linked to the hydroxyl function of very long chain (C_{30}–C_{32}) ω-hydroxyacids, which in turn are amide-linked with sphingosine. We proposed that such extended molecules could span a typical lipid membrane while the linoleate was inserted into an adjacent membrane, thus inducing the stacking of multiple intercellular membranes which is characteristic of stratum corneum. Earlier, we proposed a similar function for AGC, for which previous workers had reported attachment of the linoleate to the glucose rather than to the hydroxyacid. This difference has led us to reexamine the structures of both AC and AGC, using high field nuclear magnetic resonance spectrometry. Solutions of the lipids in deuteriochloroform:deuteriomethanol were examined at 360 MHz in a Bruker model 360 WH instrument. The spectra clearly showed that in both AC and AGC the hydroxyl groups of the ω-hydroxyacids are esterified and are liberated by the mild alkaline hydrolysis which releases linoleic acid from these molecules. Thus, AGC differ from AC only in having glucose attached to the primary hydroxyl group of the sphingosine. Since ACG are converted to AC during the conversion of granular cells to corneocytes, it can be postulated that the AGC are responsible for assembly of the lamellar bodies in the viable epidermal cells, while the AC mediate the stacking of the multiple intercellular lamellae in the stratum corneum. This is supported by our recent demonstration that AGC cause flattening and stacking of liposomes in vitro.

8. Relationship Between Sebum Secretion Rates and the Concentration of Linoleate in Sebum and Epidermal Lipids. MARY ELLEN STEWART, PHILIP W. WERTZ, MARCIA OWEN GRAHEK, AND DONALD T. DOWNING, Marshall Research Laboratories, Dept. of Dermatology, University of Iowa College of Medicine, Iowa City, IA.

There is evidence that an essential fatty acid deficiency, possibly limited to the follicle, may be involved in acne. Since acne is absent in young children until sebum secretion rates begin to rise early in puberty, we sought to determine whether changes in sebum secretion rates are associated with changes in the percentages of linoleate in sebum and whether any such changes might also be reflected in the fatty acid composition of epidermal lipids.

Skin surface lipid was extracted from the hair of 32 children, aged 6 through 9. Fatty acid methyl esters were prepared from the sebaceous wax esters, epidermal acylceramides, and cholesterol esters (of mixed origin) isolated from the extracts. These methyl esters were analyzed for lineoleate by quartz capillary gas chromatography.

In the wax esters, the % linoleate decreased from almost 3% in the lowest sebum secretors to less than 0.2% in the highest. Increased amounts of 18:2Δ5,8 partially compensated for the dilution of linoleate by larger amounts of sebum in the children with higher sebum production rates. Linoleate in acylceramides also decreased with increasing sebum production rates, the range being from about 15% to 3%. In acylceramides, linoleate appeared to be replaced by sebaceous 16:1Δ6. In cholesterol esters, linoleate first rose and then fell with sebum secretion rates. This result seemed to occur because of complex changes in chain length distribution and double bond position in the cholesterol ester fatty acids. The results support the hypothesis that changes in sebum linoleate concentration may be involved in acne.

9. Ultrastructural Features of Merkel Cells in Explanted Vibrissae. B. M. NICHOLS AND M. BELL, Depts. of Dermatology and Anatomy & Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH.

Although Merkel cells are considered to be mechanoreceptors, their relationship to cutaneous sensation is far from clearly understood. The present study was undertaken to determine conclusively the effects of denervation on this distinctive cell type.

Vibrissae from donor DBA/2 mice were excised and implanted subcutaneously in recipient DBA/2 mice. Fifteen recipient mice were anesthesized with nembutal, and their backs shaved with clippers and then with a razor. Donor mice were sacrificed; their vibrissal pads were removed and cut into blocks containing 1–3 vibrissae. The blocks were then implanted between the skin and body wall of recipient mice. Animals were sacrified at post-operative intervals of 1, 2, 3, 4 and 6 weeks, and their subcutaneous grafts were removed and prepared for examination with electron and light microscopy. Control tissues were vibrissae excised from snouts of DBA/2 mice and immediately processed according to routine procedures for electron microscopy. In normal DBA/2 mice, Merkel cells were identified in large numbers intermingled with adjacent keratinocytes in the outer layers of outer root sheaths of vibrissae; axon terminals (neurites) abutted many Merkel cells. Merkel cells were identifiable at all stages after explanation to ectopic sites, but no mitochondria-laden neurites were detected at any post-operative intervals. Therefore, it is evident that Merkel cells can survive without innervation. More likely, their maintenance depends on other factors in their environment.

10. Beta Interferon Modulates Proliferation and Differentiation of Cultured Mouse Epidermal Cells. PAMELA J. SPERRY, JOAN M. VIGIL, AND RALPH A. JUAREZ, Biological Sciences Department, California State Polytechnic University, Pomona, CA.

Interferons generally inhibit cell proliferation and stimulate or inhibit differentiation, depending on the system examined. In this study cultured newborn CD-1 mouse epidermal cells were treated with 1, 10, 10^2, or 10^3 Units/ml of mouse beta interferon (IFN) beginning the first day after planting, then every other day.

Cell proliferation was measured by ^{3}H-thymidine incorporation into DNA; cells were pulsed for 2 hours at 2, 4, and 6 days after plating. Control cells are actively proliferating at 2 days, continue to incorporate label at 4 days, but show how levels of DNA synthesis at 6 days. At 2 days IFN-treated cells demonstrated a dose dependent decrease in cell proliferation, reaching a maximum of 50% inhibition at 10^3 U/ml IFN. AT 4 and 6 days, IFN-treated cells showed no significant differences in cell proliferation compared to control cultures.

Cell differentiation was studied by pulsing the cells with ^{35}S-methionine for 6 hours on days 4, 8, and 12; proteins were extracted in 8M urea buffer, subjected to SDS-PAGE and fluorography. Interferon stimulated the synthesis of 58 and 67 kd keratins, depending on the dose and time of exposure. One thousand U/ml stimulated synthesis of these proteins on days 4 and 8. One hundred U/ml increased their synthesis on days 8 and 12. Ten U/ml was stimulatory by day 8. One U/ml did not show stimulation of synthesis until day 12.

Thus beta interferon inhibits proliferation of actively dividing epidermal cells in culture. High concentrations of IFN stimulate synthesis of the 58 and 67 kd keratins at early stages of differentiation and lower doses exhibit stimulation at progressively later times.

11. Human Fetal Epidermal Cultures. A. T. LANE AND K. A. DAY, Dermatology Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Using explant organ culture methods, 313 feeder cells and various cell culture media, we were able to culture human fetal epidermal cells from fetuses as early as 10 wks. estimated gestational age (EGA). The following table summarizes our data:

EGA (wks.)	8–10	11–13	14–16	17–19
# fetal skin biopsies	5	8	10	9
# biopsies with + culture	1	1	3	4

Each fetal biopsy was separated into multiple small explant cultures. Positive cultures were identified by several millimeter outgrowth of keratinocytes confirmed by microscopy, rhodanile blue staining or indirect immunofluorescence. Epidermal outgrowths studied by indirect immunofluorescence showed definite presence of epidermal antigens associated with reactivity to bullous pemphigoid and pemphigus antisera and monoclonal antibodies to human epidermal keratins.

Standard epidermal keratinocyte culture conditions (DMEM & additives) were compared to standard amniotic fluid culture conditions (F-10 & additives). The DMEM media more readily supported keratinocytes while fibroblasts grew more easily in F-10 media. Epidermal separation prior to culture with trypsin, EDTA or collagenase yielded no viable keratinocytes while control organ cultures developed active keratinocyte outgrowths. One 14 wk. EGA explant was passaged once.

Human fetal epidermal organ cultures offer an experimental method to develop human fetal epidermal keratinocyte culture systems. Future studies may offer insight into the control mechanisms of *in vivo* fetal epidermal development.

12. Three-Dimensional Differentiation of Eccrine Gland Tumor Cells *In Vitro*. TAMOTSU KANZAKI, SHIGEYOSHI HAGANE, AND HIKARU ETO, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Although tissue cultured-cells are known to produce organized struc-

tures, such as hair-follicle formation of trichilemmoma cells and lumen formation of endothelial cells, such cases are still extremely rare. We successfully made a well organized differentiation by culturing a human eccrine gland tumor *in vitro*.

A primary explant culture of clear cell hidradenoma was initiated on 35 mm plane plastic dishes in Eagle's MEM with 10% FCS. No special condition, such as fibroblast feeder layers or growth factors, was used. Epithelial cells were observed propagating 5 days after the culture. Eight weeks later, these cells were confluent with a dome formation, which is a characteristic of glandular cells in culture. Subculture was done successfully (plating efficiency was 20%). An attached indivisual cell propagated slowly (population doubling time was 3 days), producing a large colony. Of great interest is arrangement of these cells, i.e., cells in the center of each colony were round in shape, but cells toward the periphery were more and more spindle in concentric patterns, as observed in colonies of endothelial cell culture. Similar structures were repeatedly produced after the next subculture. Carcinoembryonic antigen was detected in these cells and in cultured media, and a monoclonal antibody (EKH5) to eccrine gland cells reacted with these cells.

This study showed that benign eccrine gland tumor cells were able to be cultured without special culture conditions and that these cells differentiated toward eccrine glands in three-dimensional structures *in vitro*.

13. Cultured Human Epidermoid Cell Sonicate Yields Ro Positive Proteins with 36,000 MW, 66,000 MW. K. A. FRITZ, C. KOSAREK, A. M. BEYER, W. GEOGHEGAN, AND R. E. JORDAN, Dept. of Dermatology, University dof Texas Medical School, Houston, TX.

The ribonucleoprotein "Ro" (SS-A), isolated from many tissue and cell lines, is found linked to RNA's hY1-5 in human beings and is said to be located in the cytoplasm. The molecular weight (MW) as determined using splenic extract is thought to be 50–57 KD. Using cultured HEp2 (human epidermoid carcinoma) cells we have shown (1) localization of Ro within the cell by indirect IF is variable depending on fixation of cells—nuclear thread like speckled in fixed cells and diffuse cytoplasmic in unfixed cells. (2) Analysis of a HEp2 sonicate by SDS-PAGE followed by Western Blot reacted with certain HuαRo sera then HPO-RaαHuIgG revealed 1 or 2 bands (see below) with MW 36 KD and 66 KD in various sub-cellular fractions.

	HSE	HEp2 Crude	Microsomal	Nucleus N. Membrane	Plasma Membrane	Cytosol
36,000 Ro+ Band		XX	XX	XX	XX	XX
66,000 Ro+ Band	XX		XX		XX	

Human splenic extract (HSE) was used as a control and showed a line of identity when reacted with αRo serum concurrent with HEp2 extract. Two distinct Ro-like proteins are thus identified in certain HEp2 cellular locations. The nature of these proteins is not known but may represent the complete protein (66 K) and either a precursor or breakdown fragment which is antigenic for some patients.

14. Primary Cultures of Differentiating Human and Rodent Cutaneous Keratinocytes Grown at the Air/Liquid Interface. L. BERNSTAM AND I. A. BERNSTEIN, Dept. of Environmental and Industrial Health, University of Michigan, Ann Arbor, MI.

The objective of this project was to produce primary cultures of uniformly stratifying cutaneous keratinocytes which are similar to the epidermis, *in situ*, by morphological and functional criteria and which can be exposed topically to toxic chemicals in order to mimic exposure of the intact skin *in vivo*. Such cultures, derived from rat and human (newborn foreskin and adult chest) skin have been grown on collagen as substrata at the air/liquid interface by modification of a published method (Lillie, et al, Exp. Cell Res., 125, pp. 153–165, 1980). The best results were obtained when keratinocytes from the rat were plated on collagen gels consisting of a mixture (1:1 by volume) of Vitrogen 100 collagen and collagen derived from rat tail. Vitrogen 100 alone served well in the case of human cells. Attachment and proliferative capacity were greater under these conditions, than when cells were plated on a plastic surface. Cultures were grown submerged for 5 (rat) or 14 (human) days and then lifted to the surface of the liquid. Up to 20 cornified layers were seen in lifted cultures cultivated 14 (rat) and 21 (human) days at the surface. Desmosomes, tonofilaments and keratohyalin-like granules were observed in these cultures. The topography of the cultures' surface was suitable for toxicological studies of "topically-applied" environmental chemicals. Biochemical characterization of the culture is in progress. Supported by USAMRDC, Contract DAMD17-82-C-2198 and Procter and Gamble Co., Contract UM-N-2.

15. Abnormal Cell Division and Differentiation of the Cultured Cell from Tuberous Sclerosis. KAZUKIYO ONODERA, YASUMASA ISHIBASHI*, MASAFUMI SASAKI†, AND GENKI KIMURA†, Dept. of Agricultural Chemistry, University of Tokyo, Tokyo, Japan, * Dept. of Dermatology, Faculty of Medicine, University of Tokyo, Tokyo, Japan, and † Institute of Bioregulation, Kyushu University, Fukuoka, Japan.

We have established a cell line from skin of patient with tuberous sclerosis and cultured the cells for 18 months *in vitro*. The cell growth was investigated using DNA binding fluorescence dye. Usually the cells divide into normal daughter cells and frequently the cells divide into a daughter cell which can divide again and an abnormal cell which has disintegrated nucleus and is unable to divide further. The cells are shown to synthesize tublin, actin, vimentin and fibronectin as fibroblasts. In addition, the cells are synthesizing microtubule associated protein 2 and glia specific protein. Profiles of cyclic AMP binding proteins are similar to those of neuron cells and differ from those of fibroblasts. The results imply that the regulation of cell division and gene expression was impaired in this cell line. Further biochemical analysis will help us to understand molecular defects associated with tuberous sclerosis.

16. Stimulation of DNA Repair by Sodium Butyrate in Xeroderma Pigmentosum Group F Cells. CHIKAKO NISHIGORI AND HIRAKU TAKEBE, Depts. of Dermatology and Experimental Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Xeroderma pigmentlsum (XP) group F, which was originally described in Japan, is characterized by late onset and mild clinical manifestations. XP F group cells has been reported to show slow and long-lasting DNA repair, which is considerably different from that of XP cells belonging to other complementation groups with some residual repair activity. Recently sodium butyrate has been reported to stimulate DNA repair capacity in human cells. We investigated whether the residual capacity in XP F group cells might be stimulated by this compound. XP cells were preincubated with various concentrations of sodium butyrate (0–20 mM) for 48 hrs. Colony forming ability and unscheduled DNA sunthesis (UDS) after UV irradiation (10–20 J/sq m) were assayed. For the source of UV light, 15W germcidal lamp was used. The amount of UDS was analysed by means of autoradiography. XP cells belonging to the complementation group F showed effective stimulation of repair after UV irradiation both in colony forming ability and UDS. UDS in XP cells belonging to the complementation group A were not enhanced, while normal cells showed enhancement, but less effectively than XP F cells. In XP A, XP C, and normal human cells, sodium butyrate treated cells showed more killing effect by UV than untreated ones. The residual repair capacity in XP F cells appeared to be stimulated by sodium butyrate.

17. A Supopulation of Cells Blocked in S Phase in Guinea-Pig Epidermis? PIERRE VAIGOT, CHANTAL DELESCLUSE, AND MICHEL DARMON, Dept. of Cell Biology, Centre International de Recherches Dermatologiques (C.I.R.D.), Valbonne, France.

DNA-synthesizing cells can be identified by incorporation of Bromodeoxyuridine (BrdUrd) followed by a staining with an FITC-conjugated anti-BrdUrd monoclonal antibody. This new procedure is analogous to autoradiography in its principle but is more accurate and simpler to perform. It can be readily combined with a total cellular DNA staining using propidium iodide as a fluorescent probe. A double-fluorescence flow cytometric analysis of bivariate DNA/BrdUrd distributions can be obtained.

BrdUrd was locally injected in guinea-pig ears each 4 h during 24 h. Animals were sacrificed after 4, 8, 12, 16, 20 and 24 h. Epidermal cell suspensions were prepared from ears and the DNA/BrdUrd flow cytometric analysis was then performed. This procedure allowed us to characterize independently 1) DNA synthesizing cells 2) cells having a DNA content comprised between 2C and 4C (classically considered to belong to the S phase). Surprisingly, this latter population was found to contain a certain number of cells (approximately 20%) which had not incorporated BrdUrd. These cells, although apparently in S phase, can thus be considered as being unable to synthesize DNA (at least during the 24 h experiment). The kinetic analysis shows that this population remains constant when the time of exposure to BrdUrd is increased from 4 to 24 h.

These results are compatible with the existence in guinea-pig epidermis of a population of cells blocked in S phase, or cycling extremely slowly.

18. Oxygen Modulates the Proliferative Lifespan of Human Fibroblasts. A. K. BALIN, S. LUSTBERG, I. LEONG, AND D. M. CARTER, Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY.

We examined the influence of oxygen tension and seeding density on cumulative population doubling level at phase-out for human diploid fibroblasts serially subcultivated at 5 seeding densities between 10^2 and 10^4 cells/cm^2 at PO_2s 13, 39, 76, 138, 210 mm Hg. We studied fetal and adult skin and lung fibroblasts. Attachment counts and atmospheric oxygen measurements were made on sister flasks at 24 hours after seeding. A Coulter Counter was used for cell counts. Cells were grown in DMEM with 10% FBS, supplemental vitamins and glutamine. We defined phase-out as inability to achieve an increase in cell number over number inoculated after 4 feedings over a 4-week period. Data are expressed as cumulative population doubling level at phase-out at the experimental PO_2 and seeding density divided by the CPDL of control cells at 20% O_2 and 10^4 cells/cm^2 × 100.

Representative data for the relative lifespan of fibroblasts serially subcultivated at 10^4 cells/cm^2 are:

PO_2 (mm Hg)	Fetal lung	Fetal skin	Adult lung
13 ± 5	165 ± 38 (6)	148 ± 34 (3)	284 ± 26 (2)
39 ± 4	185 ± 15 (2)	143 (1)	584 ± 180 (2)
76 ± 5	141 ± 19 (4)	184 (1)	576 ± 372 (2)
138 ± 13	100 (6)	100 (3)	100 (2)
210 ± 11	82 ± 1 (2)	63 (1)	43 ± 60 (2)

Physiologic oxygen tensions modulate the growth and lifespan of human cells. Serial subcultivation at PO_2s 13–76 mm Hg extends proliferative lifespan. The source of the fibroblast and seeding density appear to influence modulation of cellular lifespan by oxygen.

19. Elastase Activity in Granulomatous Inflammation in Experimental Murine Leprosy. PAIR-SHIN HSU, SEIICHI IZAKI, TOSHIHIKO HIBINO, AND MASAKATSU IZAKI, Dept. of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan.

In order to investigate elastolytic enzymes in granulomatous inflammation, murine lepromas from C57BL/6N mice infected with 10^8 Mycobacteria lepraemurium were extracted with 50 mM Tris-saline, pH 7.5, (soluble fraction). The precipitates were re-suspended in 2 M KSCN containing 0.1% Triton X-100, sonicated, and frozen-thawed to obtain bound fraction.

Elastase activity assayed with Suc-$(Ala)_3$-pNA(AAApNA), Glu-Pro-Val-pNA(s-2484), and ^{3}H-elastin, revealed that elastase activity is in the bound fraction but not in the soluble fraction; whereas a regulatory inhibitor for elastase was found in the soluble fraction. Sephacryl S-200 gel chromatography and the following DEAE-Sepharose chromatography demonstrated three fractions of elastase activity (F-I, II, and III) with distinct enzymologic properties. F-I showed a high elastolytic activity for ^{3}H-elastin, and this was totally inhibited by iodoacetamide, revealing characteristics of a thiol proteinase. This enzyme fraction did not show hydrolytic activity for AAApnA or s-2484. F-II demonstrated the highest elastase activity for all substrate. This enzyme fraction was inhibited by DFP, soybean trypsin inhibitor, and mouse serum, but not by aprotinin, elastatinal, antipain, chymostatin, or 1,10 phenanthroline, indicating this enzyme belongs to a serine proteinase unrelated to granulocyte and macrophage elastases. F-III showed a high activity for H-elastin and s-2484, as well, but not for AAApNA. Inhibitor profile was similar to F-II, suggesting that F-III is a serine proteinase unrelated to known elastases, as well.

Possibly unique types of elastolytic enzymes were demonstrated.

20. The Follow-up Study of Virus-specific Antibodies in Children with Molluscum Contagiosum. MICHIKO KAWAKAMI, Dept. of Dermatology, Tokyo Women's Medical College, Tokyo, Japan.

Molluscum contagiosum (MC) is a common viral dermatosis among children, but little is known about its immunity. In last year's JSID meeting I already reported about an indirect immunofluorescent study of MC and its results that MC virus-specific antibodies were detected in all MC patients but not in the control group without any history of MC. Among antibodies, IgG antibody was predominant, and the titer of IgG was remarkably high in patients with inflammatory MC.

The purpose of this study is to follow up the virus-specific antibody titers in MC children after treatment. By using indirect immunofluorescent study, I detected and titered the virus-specific-immunoglobulin (G, A, M, D, E) at least twice for each patient, before and after treatment. The results were as follows; IgG titer gradually decreased after treatment in all cases tested. For example, the patients with inflammatory MC showed a high mean MC virus-specific IgG titer of 320 before treatment, but after treatment it was lowered to a mean of 80 within one month. And the patients with non-inflammatory MC showed a mean IgG titer of 20 before treatment, and lowered to 10 after treatment. While IgG remained for a long time after treatment, though in a low titer, IgM disappeared within one or two months. Thus, in MC like other viral infection, virus-specific-IgM appeared only in the early phase of infection.

21. *Propionibacterium acnes* Resists Killing and Degradation by Human Leukocytes. G. F. WEBSTER, J. J. LEYDEN, R. L. MUSSON, AND S. D. DOUGLAS, Depts. of Dermatology and Pediatrics, University of Pennsylvania, Philadephia, PA.

The interaction between *P. acnes* (PA) and phagocytes is a major determinant in the resolution of acne lesions. We studied the ability of normal human neutrophils (PMN) and monocytes (MN) to kill and degrade PA in vitro. After a 2 hour incubation of 2 strains of opsonized PA with PMN no bactericidal activity was detected. In contrast, these PMN killed 100 ± 0 %log of M. Lysodeicticus and 45 ± 5.7 %log of S. aureus; MN killing of these organisms was similar. When anti-PA antibodies were present, MN killed 25 ± 26.1 and 37 ± 27 %log of PA. PA strains were rapidly killed by purified myeloperoxidase-H_2O_2-Cl at pH 5 or 6, but not at pH 7 or in the presence of azide. H_2O_2 alone, lysozyme, chymotrypsin, human serum, PMN cell and granule lysates all failed to kill PA. Degradation of PA was assessed turbidimetrically and by release of ^{14}C from PA grown in ^{14}C-glucose. Four PA strains were degraded <10% in 24 hours by lysozyme and PMN lysate. By contrast, lysozyme and PMN lysate completely lysed M. Lysodeicticus within 1 hour; S. aureus was 86% degraded and S. epidermis was 63% degraded in 24 hours by PMN lysate. Sephadex G-50 chromatography of compounds released from PA by PMN lysate revealed a peak of ^{14}C which was not released by untreated PA, suggesting that PMN enzymes do attack PA. Elution profiles for other strains incubated with PMN and MN lysates were identical. Compounds released from ^{14}C-glucose and ^{3}H-amino acid labeled PA co-eluted on G-50 suggesting that glycopeptide may be released by PMN enzymes. These data indicate that PA is resistant to the bactericidal and degradative actions of PMN and MN which may in part account for the persistance of inflammatory acne lesions.

22. The Effect of Occlusive Dressings on the Multiplication of Two Wound Pathogens. D. A. MARSHALL, P. M. MERTZ, AND W. H. EAGLSTEIN, Depts. of Dermatology, University of Pittsburgh, Pittsburgh, PA.

Proliferation of anaerobes in wounds may be a hypothetical complication of therapy with oxygen impermeable occlusive dressings. We studied the effects of occlusive dressings with varying oxygen permeability on growth of two wound pathogens, *Staphylococcus aureus* (a facultative anaerobe) and *Clostridium perfringens* (an anaerobe). Wounds (5 × 7 × 0.6 mm) were made on two pigs using an electrokeratome, and then swab-cultured for the presence of organisms as a control. One animal's wounds were inoculated with 10^8 colony forming units (CFU) of *S. aureus*; the other with 10^6 CFU of *C. perfringens*. There were six wounds per animal in each of four treatment groups—DuodDerm™, OpSite®, Vigilon™, or air exposure. Two wounds from each group were cup-scrubbed for quantitation at 24, 48 and 72 hours. All control swab-cultures were negative. *S. aureus* was recovered from all inoculated wounds. There were no significant differences in numbers of *S. aureus* recovered from any of the treatment groups. Differences were seen in recovery of *C. perfringens*:

Wound Treatment	Reported O_2 Permeability	*Clostridium perfringens* Log CFU/ml		
		24 hours	48 hours	72 hours
Air Exposure		3.0 ± 1.1	2.4 ± 0.5	2.0 ± 0.5
DuoDerm	no	6.6 ± 0.2	6.5 ± 0.0	6.6 ± 0.4
OpSite®	yes	4.0 ± 0.2	4.3 ± 1.3	6.9 ± 0.1
Vigilon™	yes	5.4 ± 0.4	6.2 ± 0.3	6.1 ± 0.7

We conclude that the varying oxygen permeabilities of different occlusive dressings do not influence growth of wound pathogens.

23. Biochemical Characterization of a Stratum Corneum Antigen and Localization to the Cell Envelope. PAMELA C. JONES, VIJAY KUMAR, EARL J. BERGEY, STEPHEN J. MILLAR, AND MICHAEL J. LEVINE, Dept. of Microbiology and Oral Biology, University of Buffalo, SUNY, Buffalo, NY.

An antigen of the stratum corneum extractable from callus with trypsin, phenol-water has been previously shown to react with autoantibodies in normal human sera (Krogh, H. K. et al. Int. Arch. Allergy *42:* 493, 1971) and with antibodies in acid eluates of psoriatic scales (Kumar, V. et al. Ann. NY. Acad. Sci. *420:* 361, 1983). This crude antigen extract was further purified and subjected to biochemical characterization and localization studies.

The active fraction of the antigen was separated on an AcA 202 gel filtration column, characterized biochemically and found to contain protein and carbohydrate, but no lipid. The major carbohydrates were galactose, glucosamine and galactosamine. Periodate oxidation partially destroyed antigen activity as tested by hemagglutination inhibition. This reaction was also inhibited by N-acetylgucosamine and by absorption of the antigen with two lectins, wheat germ agglutinin and Ulex Europaeus agglutinin I. These results suggest that at least one determinant of the antigen is carbohydrate. The major amino acids in the antigen were glutamic acid, aspartic acid, serine and glycine. The analysis had several similarities to amino acid analyses of cell envelopes isolated from callus. Cell envelopes isolated by three methods were able to absorb serum antibodies directed to the stratum corneum antigen. The presence of ϵ-(γ-glutamyl)lysine crosslinks, which are characteristic for cell envelopes was demonstrated in the antigen by the indirect cyanoethylation technique. These findings suggest localization of the antigen to the cell envelope.

24. Analysis of Keratin Compositions of Psoriatic Epidermis and Scale by Two-dimensional Gel Electrophoresis. S. DEKIO, G. J. STEVENS, K. HASHIMOTO, AND J. JIDOI, Dept. of Dermatology, Wayne State University, Detroit MI, VAMC, Allen Park, MI, and Dept. of Dermatology, Shimane Medical University, Izumo, Japan.

Keratin compositions of involved psoriatic epidermis (IPE), uninvolved psoriatic epidermis (UPE), epidermis of a normal individual (NE) and psoriatic scale (PS) were analyzed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The keratins of UPE and NE were both composed of components having molecular weights (MWs) of 67 kd to 46 kd and keratin compositions of these two samples on 2D-PAGE were identical. In IPE, 67 kd and 50 kd keratins were decreased, 52 kd keratin was increased, and some low MW components not seen in UPE were present. Although some low MW components not seen in UPE were present in IPE, all of the keratin components having MWs of 67 kd to 46 kd in IPE were seen in UPE. The reduction of 50 kd keratin in IPE was shown to be due to the lacking of acidic 50 kd components present in UPE on 2D-PAGE. Of three PS samples examined, one had only low MW components. The other two had not only components having MWs of 67 kd to 46 kd present in IPE, but also 60 kd and 54 kd components not present in IPE and many low MW components. Since the 60 kd and 54 kd components were not seen in IPE and UPE, they might be formed by aggregation of lower MW components or by degradation of higher MW components. The diversification of the low MW components in IPE and PS suggested that they were formed by degradation of higher MW components.

The present study has shown that in psoriatic epidermis, abnormal keratins were not synthesized, but the amounts of some keratins such as 50 kd components were unusually variable.

25. Monoclonal and Polyclonal Antikeratin Antibodies Probe Ultrathin Sections of Keratinocytes Prepared Via Low Denaturation Embedding. KENNETH D. MARENUS, THOMAS I. DORAN, PAUL R. BERGSTRESSER, AND DEREK R. HIGHLEY, Mary Kay Cosmetics and Dept. of Dermatology, UTHSCD/Southwestern Medical School, Dallas, TX.

Using an approach to tissue preparation for electron microscopy that is designed to preserve native protein conformation, we have obtained specimens that were stained with both polyclonal and monoclonal antikeratin antibodies. Ultrathin sections stained with polyclonal antikeratin revealed an average label density of 39.6 Au particles/μm^2. Specimens prepared via conventional embedding protocols (OsO4 fixation/acetone dehydration/heat polymerization) and stained with antikeratin antibody, presented a much lower labeling density (4.6 Au particles/μm^2). For low denaturation embedding, human skin specimens were crosslinked with 1% glutaraldehyde for 15 minutes, partially dehydrated in 100% ethylene glycol for 3 minutes and then suspended in a mixture of Vestopals. Polymerization was accomplished with low wattage, long wave UV. Ultrathin sections were then post stained with Protein A ~ Au and an aqueous solution of uranyl acetate. Specificity of labeling was demonstrated by the coincidence of label with large amorphous patches in the cytoplasm that correspond to accepted intracellular locations of keratin. Aside from low level background, gold particles were not observed over the dermis, nuclei, mitochondria, desmosomes or intercellular spaces. The ability to bind antikeratin antibodies to the surface of ultrathin sections indicates the conservation of that protein conformation which is required for antibody binding. We conclude that low denaturation embedding permits the immunolocalization of intracellular proteins and reveals an ultrastructural morphology which may be closer to native.

26. Lectin Labeling of Epidermis from Hairless and Athymic Nude Mice. MIRIAM M. BRYSK, JOANNE MILLER, SHU-JEN CHEN, AND SRINIVASAN RAJARAMAN, Depts. of Dermatology and Pathology, University of Texas Medical Branch, Galveston, TX.

The epidermal glycoproteins of hairless and nude mice were probed with Concanavalin A (Con A), *Ulex europaeus* agglutinin (UEA) and *Bandieraea simplicifolia* B_4 agglutinin (BSA). FITC-lectin overlays of cryostat sections gave identical fluorescence patterns for both mouse strains: Con A labeled all the viable layers, UEA the spinous and granular cells, and BSA only the basal cells; no labeling of the stratum corneum was observed. These results match published reports for hairy species of mouse and rat. In contrast, when samples of purified stratum corneum and viable cell fractions separated on Percoll gradients were analyzed by SDS-PAGE and then overlaid with iodinated lectins, all the epidermal layers, including the stratum corneum, were labeled by all three lectins. The Con A autoradiograms for the hairless mouse closely resembled those that we had previously obtained for human epidermis, while the autoradiograms for the nude mouse deviated significantly. The most striking difference is the absence in the stratum corneum of the nude mouse of a Mr 40,000 band which is the dominant feature of the stratum corneum of the hairless mouse and of man. With BSA there tended to be more bands labeled for the hairless mouse than for the nude mouse, with the descrepancy most evident at low molecular weights for the suprabasal cells and the stratum corneum. With UEA there was less of a difference. The radioactivity profiles for BSA and UEA had a lot more in common with each other than with Con A.

27. Evidence of Two Epidermal Tranglutaminases in Newborn Balb/c Mouse Epidermis. JUICHIRO NAKAYAMA, MITSUHIKO OSAKI, AND HARUKUNI URABE, Dept. of Dermatology, School of Medicine, Kyushu University, Fukuoka, Japan.

We have initiated to purify mouse epidermal transglutaminase in order to investigate the molecular mechanism of the induction of enzyme by 12-o-tetradecanoyl-phorbol-13-acetate or retinoic acid in cultured mouse keratinocytes (Yuspa, S.H. et al., Biochem. Biophys. Res. Commun. 97:700–708, 1980). Two separate peaks with enzyme activity were reproducibly observed in CM-cellulose chromatography of the crude extract of new born (hair free) mouse epidermis. Gel filtration on Sephadex G-150 of the combined two peak fractions in CM-cellulose chromatography showed single peak with enzyme activity, indicating that two enzyme activities had the same range of molecular weights (about 55,000). Polyacrylamide gel electrophoresis at pH 4.5 by the method of Reisfeld et al. showed two major bands with enzyme activities. The enzyme activity in Sephadex G-150 fraction had a pH optimum of 10.0 in glycine-NaOH buffer. Calcium ion and SH-protecting agent (dithiothreitol) were essential for the enzyme activity. The enzyme activity could be protected against heat-inactivation at 56°C in the presence of calcium. Trypsin treatment of the enzyme enhanced the activity about 3-fold, and this enhancement was blocked in the presence of trpsin inhibitor. In an attempt to further purify two enzyme activities observed in CM-cellulose chromatography separately, each enzyme peak fraction was gel filtered and combined to lysine-Sepharose 4B in the presence of casein at pH 6.0 in Tris-acetate buffer at 37°C. About 60% of the enzyme activity was then eluted from the enzyme-lysine-Sepharose 4B complex with 1M NaCl in the same buffer.

28. CA^{2+}-Dependent Cysteine Proteinase (Calpain) in Human Skin. SADAO IMAMURA AND YOSHIKI MIYACHI, Dept. of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Increasing attentions have been paid to the regulation of intracellular processes as mediated by calcium ions. Calpain is a typical intracellular non-lysosomal Ca^{2+}-dependent proteinase. Although the biological

functions of calpain are still obscure, a number of irreversible phenomena have been attributed to the action of calpain. In the present study, we have investigated biochemical properties and immunohistochemical localization of calpain in human epidermis using a monospecific antibody. Epidermal calpain I was extracted by DEAE-cellulose chromatography which was half maximally activated at 2-5 μM free Ca^{2+} and fully activated at 10 μM Ca^{2+}. Immunoelectrophoretic blotting of calpain revealed that only a single appeared between 67 kDa and 94 kDa, which corresponded to 83 kDa, when the blot was made with affinity-purified anti-calpain I 80K subunit IgG. Immunohistochemical staining of normal human epidermis showed that calpain I was found in mid- to upper-epidermis, but not in basal cells and was localized in the cytoplasm of keratinocytes. In psoriatic epidermis, the deposition of this proteinase was visualized very weakly just beneath the stratum corneum, which resulted in remarkable staining after PUVA treatment. On the contrary, in skin lesions with lichen planus, intense staining was readily observed in upper-epidermis. In this context, our assumption is that calpain may play an important role in proliferation and/or differentiation of keratinocytes. Possible ways of calcium control via calpain in epidermis mediated by filaggrin pathway and epidermal growth factor receptor will be discussed. To our knowledge, this is the first report on biochemical and immunohistochemical studies of human epidermal calpain.

29. Cystine-Rich Protein from Human Stratum Corneum as a Marker of Keratinocyte Differentiation. TADASHI TEZUKA AND MASAE TAKAHASHI, Dept. of Dermatology, School of Medicine, Kinki University, Osaka, Japan.

The existence of cystine-rich proteins in the marginal band fraction of stratum corneum cells has been reported. However, in many dyskeratotic diseases abundant cystine-rich proteins were found in the cytoplasm of stratum corneum cells after tissue specimens were stained with a thio-specific fluorescent reagent. The aim of this research effort was the extraction of cystine-rich protein from human sole stratum corneum.

Specimens of normal plantar stratum corneum were scraped with a scalpel blade, immediately frozen in liquid nitrogen, lyophilized and powdered by a Willy mill. Powdered stratum corneum was extracted in 50 mM Tris-HCl buffer at a pH of 7.3 over 24 hour period at room temperature. This was followed by centrifugation at 15,000 × g for 10 minutes. The resultant supernatant fraction was subjected to gel filtration via column of Sepharose 6B. The cystine-rich fraction then underwent thiol-propyl-Sepharose 6B column chromatography. The fraction was eluted in the presence of 20mM 2-mercaptoethanol and subjected to sodium dodecylpolyacrylamide gel electrophoresis. The protein band was physically separated and SDS was removed by Henderson's technique.

The cystine-rich protein was 14,000 dalton in molecular weight. Half-cystine, glycine and glutamic acid residues were 4.3%, 12.7% and 18.5% respectively. The antibody located on the cell membrane region of the upper part of the stratum spinulosum, stratum granulosum and stratum corneum. Data interpretations suggests this protein is buffer soluble and differs from those reported by other investigators in amino acid composition. Additionally, it could possibly be synthesized in the stratum spinulosum cells and may coat the cell surface of stratum corneum cells.

30. The Activation Mechanisms of Skin Sulfhydryl Oxidase. H. YAMADA, K. USUI, K. TAKAMORI, AND H. OGAWA, Dept. of Dermatology, Juntendo University, School of Medicine, Tokyo, Japan.

Disulfide bond formation is essential to the normal keratinization process in skin. Previously, we identified the disulfide forming enzyme (skin sulfhydryl oxidase; SSO) in rat skin, which catalyzes the formation of disulfide bonds in proteins, and then purified it to a single band (Biochim. Biophys. Acta:615, 309, 1980). Recently we showed that SSO mainly located in granular layer, and distributed in the cytosol of keratinocyte, suggesting that the conversion of sulfhydryl groups to disulfide in protein is carried out by SSO in the granular layer (J. Invest. Dermatol:371, 80, 1983). In this report, the activation mechanisms of SSO were studied by investigating the effect of divalent cations and proteases on SSO activity. The enzyme activity was inhibited concentration dependently by diethyldithiocarbamate, but not affected by either EDTA, EGTA, o-phenanthroline or α,α'-dipyridyl. The enzyme activity increased two fold as a result of the addition of Cu^{2+}, but was not increased by other divalent cations. These findings suggest that SSO requires Cu^{2+} as a co-factor for the activity. SSO prepared from living cells was activated by some kinds of protease such as trypsin, chymotrypsin and urokinase, but not affected by cathepsin D, plasmin and elastase. SSO from malpighian cells was more activated by trypsin than that from granular cells. These results indicate that inactive SSO (proenzyme) in malpighian cells are converted to active SSO by some kinds of serine protease during the autolysis process in granular layer.

31. The Keratinization of Embryonic and Fetal Human Skin in Organ Culture. CHRIS FISHER, JACKIE BICKENBACH, AND KAREN A. HOLBROOK, Dept. of Biological Structure, University of Washington, Seattle, WA.

Human fetal epidermis keratinizes in utero at ~24 weeks estimated gestational age (EGA). However, the keratinization specific keratins (65–67 Kd) are detected by immunoblot analysis at the time of the embryonic-fetal transition (Dale et al., 1984). Cultures of human embryonic (<60 d EGA) and first trimester fetal (60–80 d EGA) skin were established in order to test the potential of these tissues at different ages to keratinize in vitro. Samples of skin were trimmed into 3 mm^2 pieces and grown in organ culture on millipore filters supported by wire mesh. The cultures were fed from below with Dulbecco's MEM supplemented with 10% FCS. After various periods of time in culture, specimens were fixed and embedded for light and electron microscopy, or incubated in EDTA to facilitate separation of epidermis from dermis. The water insoluble epidermal proteins were analyzed by SDS-PAGE. Keratinization of the explants, as judged by s. granulosum and s. corneum formation, was found to occur in a reproducible, age-dependent fashion; older skin always keratinized more rapidly than younger skin. For example, 57 d skin did not keratinize until 23 days in organ culture, 67 d skin keratinized between 14 and 21 d in organ culture, and 75 d skin required less than 13 days to keratinize. Organ cultures of 53 and 47 d embryonic skin did not keratinize even by 30 d in culture. In all of the explants, however, the 65–67 kd keratins were found to appear well before (or in the absence of) morphological evidence of keratinization. These studies show that organ cultures of embryonic and fetal skin keratinize in a reproducible, age-dependent manner and suggest that this system may be valuable for the analysis of factors regulating keratinization.

32. Epidermal Cell Proliferation in Human Adult and Fetal Skin Organ Culture. J. R. BICKENBACH, J. E. OLERUD, AND K. A. HOLBROOK, Depts. of Medicine and Biological Structure, University of Washington, Seattle, WA.

Studies of epidermal cell proliferation typically involve the use of animal models or subcutaneous injections of radioactive or mitotic blocking agents into humans. Animal models have been valuable in demonstrating a population of epidermal cells which have a slower cell cycle than other proliferating basal cells and show long term retention of ^{3}H-TdR label (Bickenbach and Mackenzie, J Invest Dermatol 82:618-622, 1984). Label-retaining cells (LRCs), however, have not been demonstrated in human epidermis. For this purpose we have used an organ culture system in which the structure and differentiation of human skin can be maintained for long periods. Human fetal skin was grown for 70d at the air-medium interface and adult skin for 35d. Skin from fetuses <60d EGA increased in thickness from 2 to approximately 4 layers after 7d in culture and the periderm remained viable. Skin from fetuses >70d EGA increased in thickness to approximately four nucleated cell layers and by 7d in culture began to form cornified cell layers and the periderm appeared to die. Adult skin maintained its in vivo organization for the full 35d in culture. Both the adult and fetal epidermis had LI of 4–6% after 1 hr pulses of ^{3}H-TdR. The LI of adult and fetal skin after a 24 hr pulse of ^{3}H-TdR was approximately 25%.We also examined these cultures for LRCs after 8 and 14d in culture. Eight days after a 24 hr pulse of tritiated thymidine approximately 6% of the basal cells retained label. Two percent of the basal cells were still labeled 14d after labeling. Organ culture appears to be a dependable method for examining the morphology and cell proliferation of human epidermis. The finding of LRCs in human epidermis provides further support for the concept of epidermal stem cells.

33. DNA Polymerase Activities in Epidermis. NARIAKI KANEKO, SHUJI KUMANO, SHOJI MURAKATA, TOSHIHIRO HIDAKA, AND RYOHEI OGURA, Dept. of Medical Biochemistry, Kurume University School of Medicine, Kurume, Japan.

In our department in 1984, it was found that the free nucleotide content increased in hyper plastic epidermis induced by topical application of n-hexadecane. To study the role of DNA polymerase in the

accumulation of free nucleotides in the epidermis, the distribution of enzyme activity in the epidermal cellular layers was determined in normal epidermis. Guinea pig epidermis was separated into three layers; high density cellular layer (HDCL), middle density cellular layer (MDCL) and lower density cellular layer (LDCL), by centrifugation with Percoll, a colloidal silica gradient. DNA polymerase activity was determined by the level of incorporation of ^{3}H-dAMP into the acid insoluble fraction. In the case of normal epidermis, the DNA polymerase α was higher than DNA polymerase β in activity. The activity of DNA polymerase α was higher in the HDLC than in the other layers. However, DNA polymerase β was higher in the LDCL than in the other layers. These results suggested that polymerase α is connected with DNA replication and polymerase β is involved with DNA repair in the epidermis. In the case of hyper plastic epidermis, DNA polymerase activity did not show a distribution similar to the normal epidermis. DNA polymerase activities in nuclei of each cellular layer will be further discussed.

34. Correlation Between Fibronectin Distribution Patterns and Structural Changes in Skin Lesions of Systemic Lupus Erythematosus. KAZUHIRO KATAOKA, MASAHARU MIZUNO, AND SHOSO YAMAMOTO, Dept. of Dermatology, Hiroshima University School of Medicine, Hiroshima, Japan.

It is known that fibronectin (FN) is distributed at the dermoepidermal junction (DEJ) in skin lesions of systemic lupus erythematosus (SLE). In order to clarify the role of FN in SLE skin lesions, FN distribution patterns were compared with the structural changes at the DEJ in SLE skin lesions. Biopsies were taken from the skin lesions of 20 patients with SLE (male; 5, female; 15). In each biopsy specimen, histologic changes were examined by electron microscopy and FN distribution was investigated by indirect immunofluorescence using a specific antibody against FN. In 11 out of 20 specimens, the histologic changes at the DEJ were electon-microscopically mild, i.e. no multiplication of the basal lamina was observed. In these specimens, intense linear deposition of FN was observed at the DEJ. On the other hand, 9 out of 20 specimens showed severe histologic changes, such as multiplication of basal lamina. In these specimens, distribution patterns of FN were discontinuous and partially net-like, and the deposits of FNA at the DEJ were less intense and less dense than in the specimens which showed mild histologic changes. Although it has been reported that FN interacts with Clq, at the DEJ of SLE skin lesions the deposition of FN was not always parallel with that of Clq. In addition, the deposition of FN was more intense and more dense at the DEJ in the specimens obtained from clinically normal skin of SLE subjects than in those obtained from clinically normal skin of healthy subjects. These results suggest that FN distributed at the DEJ may be an important modulating factor in the histologic changes at the same zone in SLE skin lesions.

35. Localization and Identification of Gangliosides of Human Epidermis. J. A. SKRIVANEK, D. KING, R. PHELPS, E. SCHWARTZ, AND R. FLEISCHMAJER, Div. of Natural Sciences, SUNY Purchase, Purchase, NY, and Dept. of Dermatology, Mt. Sinai School of Medicine, New York, NY.

The intercellular lipids (neutral and sphingolipids) of the stratum corneum are believed to be important in the regulation of the permeability barrier of the epidermis. Although small amounts of gangliosides have been detected, their composition has not been described. Anti-GM1 antiserum, prepared from rabbits immunized with pure human brain GM1, was tested by immunofluorescence on frozen thin sections of human skin. Specific staining was seen on the membranes of the keratinocytes and was most extensive in the upper layers of the stratum spinosum and the stratum granulosum. In contrast, no staining was seen in the dermis. Epidermis was removed from fresh surgical specimens by blunt dissection, lyophilized, and lipids extracted with 5/5/1, chloroform (C)/methanol (M)/water (W). HC1 was added to a final concentration of 0.05N, and extracts were applied to a Sephadex LH 20 column. Gangliosides were eluted with 5/5/1, C/M/W, and acidic lipids separated on DEAE Sephadex. After base treatment and desalting on Sephadex LH 20 (95/5, M/W), gangliosides were separated from fatty acids and sulfatides on an Iatrobead silica gel column. Isolated gangliosides were separated by thin layer chromatography with a solvent of 50/40/10, C/M/O.2% CaC12. Following visualization with resorcinol, plates were scanned on a densitometer and amounts of gangliosides quantitated as % of the total as follows: GM3 (37%), GM1 (8%), GD3 (4%), GD1a (29%), GD2 (13%), and GT1b (9%). The presence of significant amounts of the polysialo species is unusual for extraneural tissue and may be of importance in the maintenance of the permeability barrier of the skin.

36. Skin and Urine Glycosaminoglycans in Pseudoxanthoma Elasticum. MARIA O. LONGAS, PETER WISCH, AND MARK G. LEBWOHL, Dept. of Dermatology, Mount Sinai School of Medicine, New York, NY.

Increase of a chondroitin sulfate isomer in lesional skin from a patient with pseudoxanthoma elasticum (PXE) has been reported. To establish its identity, glycosaminoglycans (GAG) of lesional skin from a patient with this disorder were purified, chemically quantitated and characterized. The preponderant GAG was completely degraded by Streptomyces hyaluronidase. A second GAG was exhaustively digested by chondroitinase ABC but not by chondroitinase AC. Their mobilities by 2-dimensional electrophoresis coincided with those of standard hyaluronic acid (HA) and dermatan sulfate (DS) respectively. GAG were also studied in the urine of 5 patients with PXE.

The data show that HA and DS are the preponderant GAG in PXE, lesional skin, with HA being higher than the normal controls by 33.6 magnitudes and DS by 4.8. The results from urine were different. HA concentrations were 2–9 times greater than the controls. These increments correlated with the severity of the disorder. Chondroitin 6-sulfate showed a similar trend, increasing with the severity of the disease by 8–18-folds. Chondroitin 4-sulfate and DS were more variable, showing no definite trend. Low concentrations of heparan sulfate were detected in the urine of two patients and heparin in one.

We postulate that the elevated concentration of GAG in lesional skin is associated with calcification of elastin fibers in PXE.

37. Effects of Hypercholesterolemic Lipoprotein Classes on Cholesterol Metabolism in Dermal Histiocytes. HAJIME KODAMA, KENZO ARAKAWA, JYOJI TADA, MITSUNORI IKEDA, AND NOZOMI NOHARA, Dept. of Dermatology, Okayama University Medical School, Okayama, Japan.

It is reported that cholesterol synthesis is suppressed and acid cholesterol esterase (ACE) activity and acyl-CoA:cholesterol acyltransferase (ACAT) activity elevated in foam cells. This study examined cholesterol metabolism in infiltrating dermal histiocytes in the presence of very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) that were obtained from the Watanabe heritable hyperlipemic (WHHL) rabbit and diet-induced hypercholesterolemic (HC) rabbit, and also in the presence of LDL from a normolipemic rabbit. The WHHL rabbit is a model of human familial hypercholesterolemia in which the serum LDL level is high. The HC rabbit resembles human type III hyperlipidemia because it shows increased β-VLDL concentration. Histiocyte infiltration was induced by injections of large molecular weight sodium dextran sulfate into the dermis of a normolipemic rabbit. Cholesterol synthesis was assayed by incubating the dermal specimen with ^{3}H-acetate for 24 hours, after the specimen was preincubated for 20 hours with the test lipoprotein. Cholesterol synthesis in histiocytes appeared to be stimulated by specific lipoprotein classes (IDL and LDL of WHHL rabbit and VLDL and IDL of HC rabbit) that increase in hypercholesterolemia and they probably had high affinity to histiocytes. ACE activity in the lysosome fraction and ACAT activity in microsome fraction were assayed after the dermal specimen was incubated with the test lipoprotein class for 40 hours, but no clear effects were observed. These findings suggest that hydrolysis of cholesteryl esters and reesterification of cholesterol do not occur before histiocytes transform into foam cells.

38. Synthesis of Elastic Fiber Components by Human Skin Fibroblasts. E. SCHWARTZ, M. G. LEBWOHL, AND R. FLEISCHMAJER, Dept. of Dermatology, Mt. Sinai School of Medicine, New York, NY.

The elastic fiber components, elastin and microfibrils, form in concert the oxytalan, elaunin, and mature elastic fiber. These three forms have been reported to correspond to stages of elastogenesis with the oxytalan fibers (OF) of the dermis consisting only of the microfibrils, the elastic fiber predominantly is elastin, and the elaunin fiber displaying intermediate characteristics. Specific antibodies against bovine α elastin and microfibrillar proteins (MFP) were tested by indirect immunofluorescence (IIF) on frozen thin sections of dermis. The antielastin antibody reacted with OF in the upper dermis and the elastic fibers. Therefore, the microfibrils of the OF are associated with elastin without the formation of the amorphous structure typical of elastin. Significant staining of the OF with the antiMFP antibody was also

observed. Early passage human skin fibroblasts were seeded onto glass coverslips and after 4, 11, 18, and 25 days reacted with antielastin, antiMFP, and antifibronectin antibodies and examined by IIF. Intense fibrillar staining with the antifibronectin antibodies can be seen at the earliest time point when only faint staining is seen with the antielastin and antiMFP antibodies. Increased staining with the elastic fiber antibodies is noted at 11 days while at the later time points significant staining is observed. These results suggest that the synthesis of elastin and MFP may be coordinated. Synthesis of MFP has been thought to precede that of elastin to function as a template for elastin deposition. We show that there is an early association of MFP with elastin both *in vivo* and *in vitro*. Furthermore, *in vitro* systems may be useful in studying alterations in elastic fibers seen in certain diseases such as pseudoxanthoma elasticum.

39. The Interaction of Keratinocytes and Extracellular Matrix. I. Secretion of Active Extracellular Matrix by Guinea-Pig Keratinocytes. SARA KUNIN*, BENO MICHEL, ISRAEL VLODAVSKY†, RAFI FRIEDMAN†, AND YORAM MILNER*, Dept. of Dermatology, Case Western Reserve University, Cleveland, OH, Depts. of *Biological Chemistry and †Oncology, The Hebrew University of Jerusalem, Jerusalem, Israel.

Guinea-pig keratinocytes in culture produce on the plastic surface a keratinocyte extracellular matrix (KEM) which can be exposed, free of cells, by treatment with detergents and alkali. The material thus prepared looks less complex, in the scanning electron microscope, than a matrix produced by corneal endothelial cells (CEM). Both of these served as excellent anchorage substrates for keratinocytes, and promoted about a five-fold increase in the attachment, spreading and growth of cells as compared to plastic alone. In addition, the colonies on KEM were much larger and led to complete plate cover. KEM synthesis in culture was followed by [^{35}S]methionine and [^{35}S]$SO_4^=$ incorporation, reaching a plateau value at approximately 10 days of growth (0.88 nmol methionine and 0.02 nmol $SO_4^=$ incorporated per 35 mm plates). Chemical and enzymatic modifications conducted on KEM showed that a protein is an active component for the promotion of keratinocyte attachment (lability to trypsin, pronase, glutaraldehyde and SDS treatments). However, also a glycosaminoglycan is an active component, since treatments with 0.2 M HNO_2 (cleavage of sulphated saccharides), 4 M guanidine-HCl (glycosaminoglycan extraction) or 50 mM KIO_4 (saccharide ring cleavage) reduced cell attachment capacity of KEM considerably. Ewing sarcoma (adhering to fibronectin) and Colon carcinoma (adhering to laminin) cell attachment on KEM increased as well; hence, the participation of these glycoproteins in the KEM structure is evident. Further studies are conducted on the control of KEM secretion and its composition.

40. Dopa-Oxidase Activity in Human Hairbulbs Measured by a Non-Radioactive HPLC Method. DEWAYNE TOWNSEND, DAVID P. OLDS, AND RICHARD A. KING, Dept. of Medicine, Medical School, University of Minnesota, Minneapolis, MN.

Tyrosinase (tase) catalyzes the oxidation of tyrosine to dopa to dopaquinone. Rorsman has recently developed a new technique for the quantitation of dopa oxidase (DO) activity of tase by taking advantage of the rapid reaction of dopaquinone with cystine to form cysteinyldopas (CD's). We have now adapted this technique to hairbulb samples and have measured DO and tyrosine hydroxylase (TH) activity of human hairbulbs for brown (n = 10), black (n = 3), white (n = 2), blond (n = 2), and red (n = 4) haired subjects for comparison of these two assays. DO activity was determined by quantitation of CD's by HPLC with electrochemical detection after enzyme release with Triton X-100. 5-S-cysteinyldopa (5SCD) and 2-S-cysteinyldopa (2SCD) were detected in the reaction products. TH activity was determined with tritiated tyrosine. Formation of 5SCD correlated with TH activity (r^2 = 0.8, n = 42) over the full range of haircolors (red, brown, black, blond, white) and enzyme activity, while 2SCD appeared to be formed nonenzymatically. The absolute amount of 2SCD was constant for each individual and did not correlate with hair color or TH activity (r^2 = 0.4). The formation of 5SCD was linear for 20 min while most of the 2SCD was formed within minutes and did not change with time. With white hair with no TH activity, 2SCD but not 5SCD was detected. We conclude that tase activity can be quantitated in human hairbulbs by this method, and that TH and DO are coordinate functions of tase over a broad range of hair color and enzyme activity.

41. Enhancement of Melanogenesis by the Activation of Gamma Glutamyl Transferase in Melanoma Cells. KOHZOH YONEMOTO AND AKIRA SAITOH, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

It has been suggested that gamma glutamyl transferase (GGT), an enzyme of gamma glutamyl cycle, takes part in melanin production, particularly in pheomelanogenesis. We have found acetazolamide (AZ), a potent diuretic, affects to enhance the GGT activity in tissue cultured melanoma cells without any influences in the cell growth or cell viability. In this study, we employed this experimental system to investigate the correlation between activation of GGT and synthesis of melanin in melanocytes.

Melanogenic B16 melanoma cells (F382A) cultured in MEM supplemented with 10% FCS were treated with AZ 3–4 days after they had been seeded, and then the AZ contained media were changed once 2 days later. Concentration of AZ in the medium we used was 10^{-3}M. Non-treated cells served as controls. They were collected 7 days after seeding and homogenized in 50 mM Tris-HCl buffer (pH 8.0). GGT activity in the cell homogenate was assayed spectrophotometrically using L-γ-glutamyl-p-nitroanilide as substrate. Melanin content in 2×10^7 cells was measured by absorbance at 400 nm. 4 different experiments were performed and analyzed statistically. GGT activity was $31.3 \pm 6.9\%$ higher ($p < 0.03$) in AZ treated cells compared to controls. Melanin content was also significantly higher ($28.6 \pm 8.1\%$) in the treated cells.

These results demonstrate that melanin production in the cells was enhanced by AZ which causes the activation of GGT. This experimental system is a unique model to prove an involvement of GGT in melanogenesis, and to achieve further studies of melanocyte functions.

42. Induction of Melanophore Differentiation in Flatfish Larvae by Irradiation of Ultraviolet Light. JIRO MATSUMOTO, TOYOKO AKIYAMA, AND TAKAYUKI ISHII*, Dept. of Biology, Keio University, Yokohama, Japan, and *Fukushima Prefectural Research Institute for Fisheries, Fukushima, Japan.

Frequent occurrence of anomalous pigmentation, mostly albinism, in hatchery-reared larvae of marine flatfish is becoming a serious problem for fishery industry. Diets and dim lighting adopted for their cultivation have been suspected to be possible causalities. In order to determine possible implication of ultraviolet (UV) light in melanophore differentiation as a causal factor, we irradiated varying doses of UVA (emission peak at 360 nm) and UVAB (312 nm) using larvae of a plaice (*Kareius bicoloratus*) reared under abnormality-causing conditions.

When UVAB was irradiated at a dose of 0.04 J/cm^2 on the water surface using a 50 cm-deep tank for two weeks over metamorphosis, melanophores which were characteristic to adult stages appeared in both eyed and non-eyed sides of the trunk. Irradiation with UVA at a similar dose failed in such melanophore induction. Appearance of adult type melanophores in the skins of non-eyed side of the trunk, which was distributed with a few of post-embryonic melanophores under normal condition, indicates that UVB play a role in melanophore differentiation in these fish. A presumption was made that pigment anomalies may result from an absence of adequate dosage of UVB at the critical stage of the development.

43. Immunohistochemical Identification of Melanosome-Associated Antigen(s) from Human Malignant Melanoma by Mouse Monoclonal Antibody, MoAb HMSA-2, on Formalin Fixed and Paraffin Processed Specimens. KAZUO MAEDA, KOWICHI JIMBOW, YUTAKA AKUTSU, AND KAORI YAMANA, Dept. of Dermatology, Sapporo Medical College, Sapporo, Japan.

We have recently developed 2 mouse monoclonal antibodies (MoAb), HMSA-1, HMSA-2, for melanosome (MS)-associated properties of human malignant melanoma (MM). This study presents the methods of development for MoAb HMSA-2 and, by means of immunohistochemistry, its specificity and distribution in normal and pathological tissues including various forms of melanocytic tumors. With Chloramin T labelling and SDS-PAGE, MoAb HMSA-1 and HMSA-2 were found to react with different species of MS proteins, though 2 out of 3 components were common to each other. Characteristically MoAb HMSA-2 was found to detect neoplastic melanocytes (MCs) of pigmented nevi (100%) and MM (80%) on formalin fixed and paraffin processed specimens. MoAb HMSA-2 did not react with normal MCs even on frozen sections. Reaction products with MoAb HMSA-2 were localized in cytoplasm of neoplastic MCs, including amelanotic MM cells. Pigmented nevi and MM could be differentiated by different reaction pattern with MoAb HMSA-2. MoAb HMSA-2 reactive proteins were not tyrosinase, but MS proteins and their precursor forms. None of 27 epithelial and soft tumors of skin and 14 brain tumors

showed positive reactivities with MoAb HMSA-2 except for xanthoma cells (2/3), giant cells of astrocytoma and neuro-secretory cells of teratoma in brain. All of our findings indicate that HMSA-2 is a unique MoAb which identifies and differentiates benign and malignant neoplastic MCs. It is useful for identifying malignant MCs scattered outside of tumor nests of MM, thus permitting a new modality for measuring the real tumor thickness of MM.

44. Effect of Oxygen on Pigmentation in Cultured Melanocytes. T. HORIKOSHI, D. M. CARTER, AND A. K. BALIN, Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY.

We previously demonstrated optimum growth of cultured human melanocytes at partial pressures of oxygen (PO_2) 7–34 mm Hg (1–5% O_2). In this study we have assessed the effect of dissolved O_2 on melanocyte function. Human melanocytes, seeded at 10^4 cells/cm^2, were grown in MEM with 5% FBS and 10 ng/ml TPA at PO_2 7 to 603 mm Hg (1% and 85% oxygen). Tyrosinase activity and melanin content were measured in cell extracts 14 days after seeding.

Tyrosinase activity (by the method of Pomerantz) was 300 μU/mg protein at PO_2 7–34 mm Hg. One unit of tyrosinase activity catalyzes the oxidation of 1 μMole tyrosine in one minute. At PO_2 235 and 355 mm Hg tyrosinase activity decreased to about 100 μU/mg protein. Thus, melanocytes cultured at lower PO_2s have higher tyrosinase activity.

Melanocytes were lysed in 0.1N NaOH for 2 hr at 90°C and centrifuged. Melanin content was measured and expressed as the optical density (475 nm) of a cell lysate containing 1 mg of protein/ml. At PO_2s between 6–135 mm Hg the melanin content was proportional to tyrosinase activity. At cytostatic concentrations (PO_2 235 and 355 mm Hg) cellular melanin content was somewhat higher, although tyrosinase activity was decreased. Possible explanations for these data include oxygen effects on cell division, oxidation of preformed melanin or oxygen effects upon some other part of the melanin synthetic pathway.

Low oxygen tension is favorable not only for melanocyte proliferation but also for tyrosinase activity.

45. Selective Cytotoxicity of Immunotoxin Consisting of Anti-gp87 Monoclonal Antibody and Ricin A-Chain for Human Melanoma Cells. TETSUZAN KANAMARU AND SHUEN-KUEI LIAO, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan, and Dept. of Pathology, McMaster University, Hamilton, Ontario, Canada.

This study was designed to determine whether immunotoxin prepared by linking the A-chain of ricin with a monoclonal antibody (MoAb) 140.240 specific for gp87 (p97 like) molecule could be used to selectively kill melanoma cells. IgG2a of the MoAb was colvalently coupled to affinity purified ricin A-chain through a disulfide linkage. The purity of the conjugate was revealed by SDS-PAGE on 10% slab gels stained with silver in that a diffuse band of the nonreduced conjugate detected migrating slower than the intact IgG2a could be dissociated into the heavy and light chain of IgG as well as two species of A-chain with M_r = 32 Kd and 33 Kd under reducing conditions. The antibody activity was retained in the conjugate as determined by the mixed hemadsorption assay against a panel of melanoma and nonmelanoma cell lines. The conjugate was 900–2000 times as effective as A-chain alone in inhibition of ^{35}S-methionine incorporation to three melanoma lines tested. This inhibitory activity could be blocked by preincubation of the conjugate with soluble antigen isolated from the spent medium of melanoma cells but not with that of carcinoma cells (HT-29). Complete inhibition of ^{35}S-methionine incorporation was achieved with 75 nM conjugate. The conjugate had at least 1000 times more inhibitory activity for melanoma cells than the control carcinoma cells. Such a conjugate preparation has potential for targeted melanoma immunotherapy. Whether the cytotoxicity of this conjugate can be enhanced by adding a conjugate comprising ricin B-chain linking to MoAb to a different epitope of gp87, or to a rabbit antibody to mouse IgG2a is under investigation.

46. Modification of Phenol and Catechol with Sulfur and Evaluation as Potential Source for Rational Chemotherapeutic Approach to Malignant Melanoma. SHUNSUKE MIURA, KOWICHI JIMBOW, YOSHIKO ITO, TAKAKO UEDA, AND SHOUSUKE ITO*, Dept. of Dermatology, Sapporo Medical College, Sapporo, Japan, and *Dept. of Chemistry, School of Medicine, Fujita-Gakuen University, Toyoake, Japan.

Melanin precursors are toxic to melanocytes (MCs). Phenols and catechols (CAT) cause depigmentation of skin and hair. To develop tools of rational chemotherapeutic approach for malignant melanoma (MM), we synthesized, in this study, cysteinylphenol (CP), cysteaminylphenol (CAP) and related CATs and examined their effect for inhibition of MM growth and depigmentation of hair. Phenols were modified with cystine and cysteamine to form 4-S-, 2-S-CPs and CAPs by HBr. 4-S-cysteinyl CAT, 2-S-cysteinyl hydroquinone (HQ) and 2-S-cysteinylresorcinol were made by combining cystine with CAT, HQ and resorcinol. 4-methyl CAT was combined with cysteine to form 3-S-cysteinyl CAT and 3-S-cysteinyl-5-methyl CAT by Ag_2O. Among 9 compounds, 4-S-CAP, 4-S-CP and 2-S-CAP showed a marked increase in the life span (%ILS) of B16 melanoma bearing mice, %ISL being 48.7%, 15.4% and 15.4% respectively. No significant increase of %ILS was seen in the groups treated with CAT derivatives. 4-S-CAP and 4-S-CP were much more potent for MM growth inhibition (GI) than DTIC while their 2-S-isomers were not, GI of 4-S-CAP, 4-S-CP and DTIC being 64.2%, 35.5% and 22.6% respectively. 4-S-CP and CAP formed dopachrome pigments by MM tyrosinase while their 2-S-isomers did not, Km and Vmax of 4-S-CP being 0.021 and 2.3 while those of 4-S-CAP having 0.031 and 2.3. Local injection of 4-S-CP and CAP caused depigmentation of black hair with loss of functioning MCs. Thus, 4-S-CP and CAP are melanin precursors that inhibit the growth of MM cells and MCs, disintegrate these cells and increase life span of MM bearing mice.

47. Growth Factor and Cytogenetic Abnormalities in Malignant Melanomas and Non-malignant Nevi. ANN RICHMOND, JAN FERNANDEZ, ROBERT G. ROY, CHRIS ENGEL, ROBERT FINE, DOUGLAS MURRAY, DAVID H. LAWSON, AND JEAN PRIEST, Depts. of Medicine, Surgery, and Pediatrics, Emory University and V.A. Medical Center, Atlanta, GA.

One of the earliest traits of the "pre-malignant" nevus is hyperproliferation. A direct correlation between activation of growth genes and appearance of chromosomal rearrangements has now been established for several types of neoplasms. Human melanoma cells have been shown to produce autostimulatory mitogens which stimulate serum free monolayer growth in both melanoma and nevus cultures. We have studied production of mitogenic polypeptides and appearance of chromosomal abnormalities in 3 melanoma cell lines and in cultures established from 8 nevi with normal histology and 6 malignant melanomas. Cultures were karyotyped and monitored for production of melanoma monolayer mitogens. In the nevus cultures, 5 of 8 released mitogenic activity when cultured in serum free medium. Three of the 5 mitogen positive cultures had definable chromosomal abnormalities. One nevus culture exhibited a chromosomal abnormality, but was mitogen negative. In the newly established melanoma cultures, 5 of 6 had both karyotypically normal and abnormal populations while one culture was totally abnormal in karyotype; 3 of the 6 were mitogen positive. In the 3 melanoma cell lines studied all cells were karyotypically abnormal and 2 of the 3 cell lines were mitogen positive. Though karyotype abnormalities were complex, the abnormalities of 1, 6 and 7 reported by other laboratories were noted in 7 of the 9 melanoma cultures. These data suggest that production of melanoma monolayer mitogens may be coincident with, but are not limited to appearance of definable chromosomal abnormalities.

48. Relation of HLA-DR and Melanoma Associated Antigens to Cellular DNA Content in Human Melanoma Cell Lines In Vitro: Two Parameter Flow Cytometric Analysis. KOICHIRO KAMEYAMA, SHIN-ICHIRO TAKEZAKA, AND TAMOTSU KANZAKI, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

In some cases of malignant melanomas, tumor cell show heterogeneity in terms of expression of HLA-DR antigen and melanoma associated antigen (MAA) even in a single skin lesion. In this study, we investigated

1) if the expression of HLA-DR antigen and MAA has any relation to cellular DNA content and

2) the effect of recombinant gamma interferon (r-IFN-gamma) on the expression of HLA-DR antigen in two human melanoma cell lines (A101D and KHm-¼). Melanoma cell lines were stained with monoclonal anti HLA-DR antibody (L243) or monoclonal anti MAA (96.5). Cells were then incubated with FITC conjugated goat anti mouse Ig antibody. After fixation in 70% ethanol and digestion with RNase, cells were stained for DNA with propidium iodide and were analysed in two-parameter flow cytometry for the relation between expression of cell surface antigens and DNA content. The effect of r-IFN-gamma on

melanoma cells were examined after 72 hours culture with 500 units/ml of r-IFN-gamma in the medium. Result are as follows: 1) Expression of HLA-DR antigen was increased from G1 phase to G2/M phase, however the density was virtually constant throughout cell cycle in both cell lines. 2) Expression of MAA was sharply increased at S phase and decreased at G2/M phase in A101D cells. 3) By r-IFN-gamma treatment, the expression of HLA-DR antigen was increased in both cell lines. These findings suggest that heterogeneity of MAA expression may relate to cell cycle, however HLA-DR antigen expression may not.

49. Growth Responses of Normal and Malignant Human Melanocytes. RUTH HALABAN, SIKHA GHOSH, PAUL H. DURAY, AND AARON B. LERNER, Depts. of Dermatology and Pathology, Yale University School of Medicine, New Haven, CT.

Human melanocytes derived from newborn foreskins need both the phorbol ester TPA and isobutylmethyl xanthine (IBMX) and/or cholera toxin to proliferate in culture. IBMX is superior to cholera toxin in eliciting the synergistic stimulus. Four major proteins (TPAp53, p38, p37 and p31) increase in abundance, and two major proteins (p35 and p32) decrease in abundance in response to the growth factors. In two human melanoma cell lines that can grow in the absence of TPA and IBMX, TPAp53, p38 and p37 are expressed constitutively and p35 is suppressed constitutively or expressed only at low levels. Thus, proliferating normal human melanocytes may express transformation-related proteins that are also expressed in those melanoma cells that do not need the factors to proliferate in culture. TPAp53 is, however, not immunologically related to either the SV40-induced p53 or to the c-fos protein (p55). The two proteins that are expressed in growth-arrested melanocytes may have a role in suppressing proliferation.

Melanocytes derived from congenital and dysplastic nevi and from normal skin of adults, also need TPA, IBMX and/or cholera toxin to proliferate in culture. However, these cultures have a short life span and the rate of cell proliferation is 1/10 or less of that in cultures derived from newborn foreskins. Melanocytes from some primary superficial spreading melanomas and from some secondary satellites also need TPA, IBMX and/or cholera toxin to proliferate in culture. It is possible that an early event in the malignant transformation of melanocytes is a progressive acquisition of responsiveness to growth factors such as exhibited by newborn melanocytes. The ability to proliferate independently of these factors may be a later event in malignant transformation to melanomas, such as during metastasis.

50. Characterization of Two Monoclonal Antibodies to Human Epidermal Keratohyalin. BEVERLY A. DALE, ALLEN M. GOWN, AND PHILIP FLECKMAN, Depts. of Periodontics, Medicine (Dermatology), and Pathology, University of Washington, Seattle, WA.

Keratohyalin (KH) is a distinctive morphologic component of the granular layer of epidermis. One component of KH is the protein profilaggrin (M_r > 200 kd), precursor of filaggrin (M_r 37 kd), the presumed keratin matrix protein. Partially purified human filaggrin was used to immunize BalbC mice. Hybridomas were prepared and antibody-producing clones detected by immunofluorescence on frozen sections of human skin. Two anti-keratohyalin antibodies (AKH1 and AKH2) were further characterized. Both antibodies strongly stain KH and portions of stratum corneum. They also stain cultured keratinocytes in a granular distribution in confluent cultures. On immunoblots of epidermal extracts AKH1 stains profilaggrin and filaggrin while AKH2 stains bands of 130 kd and 210 kd. However, only the profilaggrin band is detectable on blots of extracted cultured cells.

In ichythyosis vulgaris (IV) KH is absent or abnormal. AKH1 staining in IV is negative or weak, consistent with the morphology and with biochemical evidence of the absence of filaggrin. In contrast, AKH2 staining is positive in IV, although not as granular as in the normal. Staining with both antibodies is stronger than normal in lamellar ichthyosis, in which KH is present in excess. Peptide mapping and ion-exchange chromatography suggest that the 130 kd and 210 kd bands (AKH2 positive) are related cationic proteins, however, they appear to be unrelated to filaggrin and profilaggrin (AKH1 positive). These antibodies confirm the complex nature of keratohyalin and will be useful to identify KH proteins other than profilaggrin that may be markers of epidermal differentiation.

51. Establishment of a Mouse Monoclonal Antibody, MoAb HMSA-1 Against Melanosome-Associated Antigen(s) of Human Malignant Melanoma. YUTAKA AKUTSU, KOWICHI JIMBOW, AND KAZUO MAEDA, Dept. of Dermatology, Sapporo Medical College, Sapporo, Japan.

The melanosome (MS) is an organelle unique to normal and malignant melanocytes (MC). It is membrane-bound and composed of tyrosinase, structural matrix proteins and phospholipids. Our previous studies indicated that MS proteins consist of, at least, 15–16 polypeptides which are, on SDS-PAGE, either common to normal and malignant MCs or unique to either one of these. To characterize the biological properties unique to malignant MCs and to utilize these for establishing laboratory diagnostic tools of malignant melanoma (MM), we have developed mouse monoclonal antibodies (MoAb) against MSs of human MM. The isolated MSs were solubilized by non-ionic detergent, partially purified by a DE52 column, and immunized against BALB/c mice. The immunized spleen cells were fused with mouse myeloma cells of Sp 2/0 Ag14. Among 18 mother bottles which revealed positive reactivities against MS proteins of MM and blue nevi, 3B2 was cloned by a limited dilution of hybrid cells. The MoAb developed, named as HMSA-1, was of IgG subclass. The subcelluler fractionation of cell organelles indicated that the antigen(s) reactive with MoAb HMSA-1 was highly localized in fractions of MS, microsome and rough ER of human MM by an ELISA. MoAb HMSA-1 reacted characteristically with formalin fixed and paraffin processed MM and pigmented nevi. Proteins reactive with MoAb HMSA-1 were discerned on SDS-PAGE into 3 bands, none of which were, however, found to be related to tyrosinase. MoAb HMSA-1 did not react with normal human MCs, nor did it react with normal tissues and organs of fetus and adults, thus it identifying the differentiation antigen(s) of MS-associated properties in human neoplastic MCs.

52. A New Monoclonal Antibody Which Identifies Liposarcoma (LS) Cells. S. MATSUO, N. PENNEYS, J. ZIEGELS-WEISSMAN, AND K. ADACHI, Depts. of Dermatology and Pathology, University of Miami School of Medicine, Miami, FL, and Dept. of Dermatology, Asahikawa Medical College, Hokkaido, Japan.

The demonstration of antigen characteristic of a specific cell type within a tumor cell supports the concept that the tumor cell is derived from that parent cell. We are generating an array of monoclonal antibodies to tissue antigens that are stable to formalin fixation and paraffin embedding; such antigens, when present in a neoplasm, would be of help in identification and classification of the neoplasm.

Standard mouse-mouse hybridoma methodology was used after immunization with homogenate of human LS. The supernatants of viable colonies were assayed for antibody by an unlabelled antibody peroxidase-antiperoxidase method using sections of formalin-fixed paraffin embedded LS as substrate. An antibody was identified to an antigen in the cytoplasm of LS cells. Nine cases of LS, 12 of normal fat or lipoma, 3 of fat necrosis, and a variety of controls were examined at various dilutions of the primary antibody and using appropriate controls in each experiment.

The monoclonal antibody identified an intracellular antigen in atypical cells in all cases of LS including a variety of histologic types. The antigen was not detected in other well-differentiated sarcomas. The antibody detected a substance in sebaceous gland cells (4 cases) and in fat cells in areas of fat necrosis. There was weak positivity overlying normal chondrocytes in one case. The use of this antibody may facilitate the identification of poorly differentiated LS and sebaceous gland lesions.

53. Subacute Cutaneous Lupus Erythematosus (SCLE): An Analysis of Serologic Immunogenetic and Prognostic Factors. J. F. FOWLER, J. P. CALLEN, K. B. KULICK*, G. STELZER, C. MACDONALD*, AND P. COTTER, University of Louisville School of Medicine and the Veterans Administration Hospital, Louisville, KY, and *SUNY and Veterans Administration Hospital, Buffalo, NY.

Subacute cutaneous lupus erythematosus (SCLE) was clearly separated from widespread, chronic, cutaneous lupus erythematosus by Sontheimer et al in 1979. Two forms of SCLE have been recognized: annular and papulosquamous (PS) variants. Subsequent investigations have shown that anti-Ro antibodies and HLA-DR3 are present in a majority of these patients. Further, mild systemic disease is common, but the skin disease is generally the patients' major problem, and, lastly, the patients follow a relatively benign but chronic course. We have studied 49 patients with SCLE (31 white females, 12 white males, 4 black females and 2 black males). The cutaneous disease was primarily annular in 14 patients, PS in 29, and a combination in 6. DLE was present in varying degrees in 17 patients. Twenty-five patients fulfilled the criteria for the diagnosis of systemic LE. Cytoplasmic antibodies were present in only 16 patients (10 Ro, 2 La, 4 both). Presence of

these antibodies did not correlate with cutaneous disease or any other clinical feature. HLA studies documented only a slight increased prevalence of HLA-DR3 (17 patients) (p = N.S.). Clinical renal disease was present in 9 patients, 3 of whom had decreased renal function. Although the disease was active in 27 patients, 22 patients had inactive disease. Thus, our population is not as distinctive as previously suggested, the immunogenetic and serologic laboratory correlates were not clearly demonstrated, and a full spectrum of clinical disease was seen from severe systemic disease to a short-term benign cutaneous disease.

54. The Effect of a Polyethylene Oxide Hydrogel Dressing Containing Povidone Iodine on *Staphylococcus aureus* Infection in Wounds. P. M. MERTZ, D. A. MARSHALL, AND W. H. EAGLSTEIN, Dept. of Dermatology, University of Pittsburgh, Pittsburgh, PA.

We studied the ability of a polyethylene oxide hydrogel (POH) dressing containing Povidone iodine (PI) to prevent *Staphylococcus aureus* proliferation in shallow wounds. We reported previously that a single application of PI on wounds challenged with 2×10^6 *S. aureus* was not effective in reducing the number of *S. aureus* after 24 hrs. We hypothesized that continuously available PI might be effective if applied to a smaller number of bacteria in wounds. To test this hypothesis, we made multiple wounds ($5 \times 7 \times 0.3$ mm) on three domestic pigs. Wounds were challenged by scrubbing with either 10^3, 10^4, or 10^7 *S. aureus* suspension. Wounds were treated with POH + PI, POH or left air exposed. From each of these treatment groups, two wounds were cup-scrubbed for *S. aureus* with a 0.5% $Na_2S_2O_3$-Tween 80 broth at 5 min, 30 min, 24 hrs or 48 hrs. A reduction in numbers of *S. aureus* was seen with 10^3 and 10^4 challenges, but no reductions were observed when wounds were inoculated with the larger challenge of 10^7.

RECOVERY OF *Staphylococcus aureus* LOG CFU/ml

Treatment	10^3 (Log 3)			10^4 (Log 4)		
	POH + PI	POH	Air	POH + PI	POH	Air
5 minutes	0	2.2 ± 0.2	2.4 ± 0.0	1.6 ± 0.2	3.6 ± 0.2	3.4 ± 0.0
30 minutes	0	2.2 ± 0.1	2.5 ± 0.1	0	3.5 ± 0.2	3.5 ± 0.2
24 hours	0	6.6 ± 0.1	6.4 ± 0.3	5.4 ± 0.2	6.1 ± 0.9	6.4 ± 0.3
48 hours	6.3	7.4 ± 0.3	7.0 ± 0.3	5.6	7.8 ± 0.0	5.2 ± 0.4

We found POH + PI dressing to be an effective inhibitor of small numbers of *S. aureus* in wounds over a 24-hour period.

55. Ultrastructural Localisation of Carcinoembryonic Antigen (CEA) in Paget's Cell and Sweat Apparatus. A Study Using Immunoperoxidase Technique K. UEDE, T. KIRI, AND M. MATSUNAKA, Dept. of Dermatology, Wakayama Medical University, Wakayama, Japan.

Purpose: CEA in sweat gland tumor can be detected by immunoperoxidase technique, and this technique is useful for identification of subclinical triple extramammary Paget's disease which is frequently encountered in Japan. We examined the sites of reaction in Paget's not yet been made known so far. Methods: PLP-fixed, frozen sections treated with immunoperoxidase were fixed with Os, embedded in Epon, and observed with an electron microscope.

Results: Paget's cell; CEA-positive substances were noted in glycocalyx, intracellular (cytoplasm) and adjacent keratinocytes. Normal sweat gland; CEA-positive substances were found out in glycocalyx, intralumen and intracellular canaliculi. Comment: It is accepted that extramammary Paget's disease originates in sweat gland; the findings obtained lead us to consider that CEA which is cell membrane surface-orienting in normal conditions was found out within the cell after its polarity was damaged due to oncogenesis; further, another finding that CEA was also recognized on the cell membrane surface of adjacent keratinocytes suggests that an atypical differentiation occurred in this keratinocytes as well.

56. Chronic Hypersensitivity in *Propionibacterium acnes* (Pa) Induced Rat Ear Inflammation. DOREEN A. SPIRES, LARRY M. DE YOUNG, AND STEPHEN J. BALLARON, Syntex Research, Palo Alto, CA.

We have previously shown a single intradermal injection of certain strains of killed Pa produces chronic, acne-like inflammation in the rat ear. Experiments were conducted to determine if hypersensitivity to Pa could be demonstrated. Female Sprague-Dawley rats (8/group) were intradermally sensitized in the left ear with 70 μg Pa strain CN 6134 and homologously challenged in the right ear with 14 μg. Inflammation (right ear thickness) was determined after challenge. Rats showed hypersensitivity as early as 1 week and remained sensitized for at least 7 weeks. In a typical experiment, rats having a baseline ear thickness of 0.5 mm and challenged 4 weeks after sensitization, had 1 day post challenge ear thicknesses of 1.4 mm as compared to 0.9 mm for control (challenge dose only). The increase in ear thickness peaked at 195% above control 14 days after challenge, and remarkably was still 61% above control 28 days after challenge. At 35 days after challenge ear thickness was 25% above control and only at 42 days did ear thickness return to control levels of inflammation. This hypersensitivity is different from classic immediate or delayed type hypersensitivity, since the increased inflammatory response in sensitized animals is chronic rather than transient. We have found that Pa strains capable of inducing chronic inflammation are retained in macrophages for up to 26 days after a single injection, and we therefore postulate a single Pa injection could act as both sensitizer and challenger.

57. Elicitation of Organized Granulomas (Gr) in Murine Skin Without Inoculation of Viable Cells. M. OKAMOTO, K. FUKUYAMA, AND W. L. EPSTEIN, Dept. of Dermatology, University of California, San Francisco, CA.

In order to investigate basic mechanisms of granulomatous inflammation in skin, a suitable animal model is required. This paper reports an improvement on a previous model in which organized Gr were produced in the skin of nonsensitized mice by isograft of hepatic egg Gr from mice infected with *Schistosoma mansoni*. Egg Gr were surgically inoculated into 3 groups of normal BALB/c mice 1) without further treatment, 2) after freezing/thawing 3 times and 3) after freeze-drying. Grafted sites were excised 2, 3 and 4 wks later. A portion of each sample was used to histologically count the total eggs present and compute the % of organized Gr which developed, while the other portion was used for measurement of a biochemical probe, angiotensin converting enzyme (ACE). Gr began to appear at 2 wks and increased at 3 and 4 wks, with a rise of ACE activity. As summarized in the Table, there were no significant differences in Gr formation at 4 wks between the 3 groups.

	% Gr	ACE Activity (mU/mg protein)
Fresh Gr	20.5 ± 13.9 (n = 5)	12.6 ± 1.7 (n = 3)
Freezing/thawing	21.6 ± 11.6 (n = 6)	23.4 ± 13.0 (n = 3)
Freeze-drying	23.8 ± 14.1 (n = 5)	14.8 ± 3.3 (n = 3)

Inoculation of eggs alone did not produce Gr. These data indicate that inoculation of viable cells is not a necessity for Gr formation, and that constituents of Gr, other than eggs, play a trigger role in eliciting Gr. Characterization of these factors is expected to contribute to an understanding of mechanisms of organized granuloma formation.

58. Cyclic Endothelial Cell Injury and Chronic Blood Vessel Wall Alterations in Familial Cold Urticaria. M. G. TONNESEN, R. A. F. CLARK, S. L. SIEGAL, B. R. REED, L. CAPIN, AND A. A. WANDERER, Depts. of Dermatology, Pediatrics, and Medicine, National Jewish Hospital, VA Medical Center, and University of Colorado, Denver, CO.

Familial cold urticaria (FCU) is a rare autosomal dominant disorder characterized by the development of rash, fever, arthralgias and neutrophilic leukocytosis, following generalized exposure to cold. Biopsies of typical skin lesions have revealed marked perivascular neutrophil (PMN) infiltration. In order to delineate further the clinical and histopathological evolution of the cutaneous eruption in FCU, a 56 yr. old female who has suffered from daily episodes of FCU since birth was studied during an acute flare induced by exposure to cool ambient air (13–18°C) for 3 hours. Lesions began as erythematous macules within one hour following cold exposure, then developed into papules which coalesced to form plaques. Skin biopsies of lesions were obtained at 0, 5, 7, and 10 hrs. and examined both by 1 um section and immunofluorescence techniques. Pre-cold exposure biopsy of non-lesional skin revealed a mild mononuclear cell perivascular infiltrate, focal endothelial cell (EC) activation and injury, and striking perivascular fibrosis. Anti-laminin staining demonstrated basement membrane thickening. As lesions evolved, progressive neutrophil influx occurred. Progressive microvascular damage was evidenced by EC swelling, smudging, and vacuolization with near total luminal obliter-

ation in the papule stage (without red cell extravasation or nuclear dust), followed by focal re-establishment of patent vascular channels in plaque lesions. In addition, EC injury was manifest by focal loss of granular factor VIII staining with perivascular diffusion. Findings were confirmed on biopsy of a typical lesion in a second but unrelated patient during an acute flare of FCU. The pathogenesis of the cutaneous eruption in FCU, a genetically determined recurrent acute inflammatory disorder, appears to be associated with cyclic recurrent acute injury and chronic repair processes of the microvasculature.

59. Androgen Dependency and Receptor Characteristics of Pilosebaceous Tumor Developed in *Suncus murinus*. SUSUMU TAKAYASU AND SATOSHI ITAMI, Dept. of Dermatology, Medical College of Oita, Oita, Japan.

A pilosebaceous tumor spontaneously developed on the sidegland of male *Suncus murinus* has been serially transplanted to male nude athymic mice for 23 generations. Since transplantability was lowered (from 90 to nearly 60%) in later passages, we studied some characteristics of the androgen receptor (AR) of these relatively androgen-insensitive tumors. First, saturation analysis by an exchange method revealed an appreciable amount of cytoplasmic AR (40–190 fmol/mg protein), while nuclear AR levels were significantly lower than those in the earlier passages (<50 vs 50–430 fmol/mg protein). Second, gel-filtration through Sephadex G-25 revealed that less than 20% of the radioactivity in the nuclear extract was bound to macromolecules 1h after the injection of [^{3}H]-testosterone. In contrast, in the tumors of earlier passages actually all of the radioactivity was in the bound fraction. Besides, on sucrose density gradients, the cytoplasmic fraction labeled with [^{3}H]-R1881 showed a broad shoulder as well as a 9S peak. In contrast, the hydroxylapatite assay of the postlabeled gradient fraction produced a sharp peak at 9S with a minimal shoulder. Therefore, the broad shoulder obtained by prelabeling is considered due to the dissociation of [^{3}H]-R1881 from AR during ultracentrifugation. These data suggest that the lower affinity of cytoplasmic AR to androgen and disorders in nuclear translocation of androgen-receptor complex cause a loss of androgen sensitivity of the tumor.

60. Effects of Low-Output Carbon Dioxide Laser Irradiation on Wound Closure and Healing Following Scalpel Incision. J. GARDEN, J. ROBINSON, M. TAUTE, J. LEIBOVICH, AND E. LAUTENSCHLAGER, Northwestern University, Chicago, IL.

Cutaneous wound healing of scalpel induced incisions to the depth of adipose tissue closed with conventional methods was compared with low-output carbon dioxide (CO_2) laser irradiation closure. Three Pitman-Moore minipigs had four rows of five 2.5 cm scalpel incisions placed over each half of their backs. Wounds were closed with either subcutaneous and epidermal sutures, subcutaneous sutures and epidermal staples, epidermal staples followed by CO_2 laser irradiation, or subcutaneous sutures followed by CO_2 laser. Laser output was 500 mW with a 0.8–1.0 mm diameter spot size for two of the three pigs. The spatially averaged energy fluence for each incision was 60.3 ± 7.3 J/cm^2. The initial pig received 3–4 times this total energy. Tensile strength and histological studies were done on days, 1, 2, 3, 5, 7, 9, 14, 21, 42, 56 and 90.

According to clinical parameters, all wounds closed and healed well. Tensile strength studies, excluding the laser irradiated areas of the initial pig, revealed no significant differences with any of the modalities. Histologically, the initial eschar and clot formation were similar in all wounds; however, the laser irradiated areas also had thermal changes of the epidermis and papillary dermis for an average of 0.6 mm on each side of the wound. No significant differences in the regeneration of the epidermis, neovascularization, or elastin and collagen formation resulted among the various modalities of closure. However, the initial pig had extensive thermal damage in laser irradiated areas with decreased initial tensile strengths and large eschar formation.

The results indicate that low-output CO_2 laser energy may be used in wound closure in a manner equivalent to that achieved using conventional methods allowing its use in wound healing study.

61. Conjugation of Topically Applied Phenol During Passage Through the Skin of Newborn Mice. JEANNETTE M. BONIFAS, ADAM EPSTEIN, AND ERVIN H. EPSTEIN, JR., Dept. of Dermatology, San Francisco General Hospital and the University of California School of Medicine, San Francisco, CA.

Conjugation of lipophilic molecules to more hydrophilic metabolites is a well-studied mechanism by which the body detoxifies noxious substances. Such conjugation occurs not only in the liver but also during absorption in the gut. Cultured keratinocytes and epidermal homogenates do contain conjugating enzymes but there are few methods for determining whether conjugation actually occurs during transcutaneous passage of topically-applied chemicals. We have studied this process in a simple in vitro system.

Fresh skin from 3-day-old mice, after removal of subcutaneous fat, was clamped in a plastic chamber designed for percutaneous absorption studies. The lower well was filled with Eagle's MEM. ^{14}C-phenol was applied in 20 ul of acetone to the dry upper surface. After 4 hr at 37°, aliquots of the medium in contact with the dermal side were applied to silica gel TLC plates, which were developed with butanol: water:ammonium hydroxide (10:1:1). Both phenyl sulfate and phenyl glucuronide were formed, but neither was present if the skin were frozen and thawed before use.

We conclude that skin can conjugate substances during their passage from the stratum corneum into the dermis. This provides a second line of defense against chemicals which the stratum corneum is unable to exclude completely.

62. Nuclease Digestion Analyses of Epidermal Chromatin of New Born and Senescent Rats. PRAKASH C. SUPAKAR AND GOPAL M. BHATNAGAR. Dept. of Dermatology, The Johns Hopkins University School of Medicine, Baltimore, MD.

Chromatin is organized into a repeating structure (nucleosome) made up of protein and DNA. We have used micrococcal nuclease and DNase I to probe this structure in epidermis of newborn (2–3 days) and senescent (24 months) Wistar rats. Epidermis was separated from dermis and homogenized to obtain nuclei by differential centrifugation. The crude nuclei was filtered through a series of nylon monofilaments. Further purification was acheived by centrifugation through high density sucrose gradient. The purified nuclei were digested with micrococcal nuclease or DNase I and at different times the extent of DNA digested was measured. Purified liver nuclei were used for comparison. The rate of DNA digestion show an initial fast rate in which 10% of DNA is digested into soluble fraction, followed by a slow phase that results in a maximum digestion within 5 min in epidermis and 30 min in liver. The extent of DNA digestion by DNase I in liver nuclei is about twofold greater than that of newborn epidermis and about fourfold higher when compared to 24 mo epidermis. There is no significant age-dependent variation in the susceptibility of DNA to micrococcal nuclease, however an age-related inhibition of DNA digestion by DNase I in older rats is found. These results indicate a higher gene activity in newborn rat epidermal chromatin and probably an age related masking by nucleosome core histones which limits the accessibility of DNA to DNase I in older rats.

63. Pharmacological Characteristics of the Cutaneous Vasopermeability Action of Various Venoms. JOSEPH W. BURNETT AND GARY J. CALTON, Div. of Dermatology, University of Maryland School of Medicine, Baltimore, MD.

An attempt to pharmacologically counteract the vasopermeability inducing action of venoms from a wasp (Vespa orientalis), an anemone (Bolocera tuediae) and three jellyfish (Chironex fleckeri, Chrysaora quinquecirrha, Physalia physalis) was conducted by administering salicylates, methysergide, diphenhydramine, ibuprofen, indomethacin, cromolyn, hydrocortisone, cimetadine, vinblastin, a serotonin receptor antagonist (LY53857), and a leukotriene inhibitor, piripost (U-60257). Results were calculated by the Evans blue dye technique in albino rats measuring the diameter of the discoloration at the site of venom challenge. Piripost treated rats significantly had smaller blue spots if the drug was administered 4–5 min prior to challenge with any of the 3 jellyfish venoms. Chrysaora venom, whose ability to extravasate dye was reduced 80% by prior piripost therapy, did not appear to interfere with lipoxyginase activity in either leukocytes or platelets. Methysergide counteracted the cutaneous bluing induced by the venom of all 4 coelenterates, whereas; cromolyn, indomethacin, ibuprofen and cimetadine were effective inhibitors of the action of Chironex venom. None of these inhibitors prevented the leakage induced by Vespa venom, and hydrocortisone enhanced it. These studies give a clue to the preferred pharmacological agent to protect the skin from the action of these venoms, and also demonstrate that these venoms have different actions on cutaneous vasopermeability.

64. Activation of Phospholipase A2 Induced Decreased Responsiveness of PGE- and β-Adrenergic-Adenylate Cyclase of Pig Epidermis. HIDEKO SUYA, TAKASHI AOYAGI, AND YUSHO MIURA,

Dept. of Dermatology, Hokkaido University School of Medicine, Sapporo, Japan.

Involved psoriatic epidermis has been characterized by decreased responsiveness of PGE- and β-adrenergic-adenylate cyclase responsiveness. In order to induce decreased adenylate cyclase responsiveness, we attempted to stimulate phospholipase A2 activity and to examine adenylate cyclase responsiveness of pig epidermis. There were no significant alterations of basal level of cyclic AMP following the EGF treatment. EGF induced decreased PGE- and β-adrenergic-adenylate cyclase responsiveness, although no altered responsiveness of histamine- or adenosine-adenylate cyclase was obtained. Decreased PGE-adenylate cyclase responsiveness induced by EGF was completely eliminated by the addition of indomethacin to inhibit PGs synthesis. It is considered that activation of phospholipase A2 by EGF induced refractoriness of PGE-adenylate cyclase, but the precise mechanism to induce decreased β-adrenergic adenylate cyclase by EGF was not fully documented.

65. Retinoic Acid Induces Cyclic Changes in Epidermal Cell Kinetics and Dermal Synthesis of Collagen and Glycosaminoglycans. J. SCHILTZ, J. LANIGAN, B. PETTY, W. NABIAL, AND J. BIRNBAUM, American Cyanamid Company, Clifton, NJ.

The effects of daily topical application of retinoic acid (RA) on guinea pig ear skin was studied. After daily treatment with 0.05% RA, the epidermis became hyperplastic with marked psoriasiform histological features. The hyperplasia developed after 6 days and resulted directly from a transient 4-fold increase in epidermal basal cell replication on days 2 and 3 (^{3}H-TdR autoradiography). Basal cell activation and limited hyperplasia occurred in guinea pig skin cultured in vitro as organ explants; epidermal cells in monolayer culture became enlarged, postmitotic and developed extensive keratin filaments characteristic of fully-differentiated keratinocytes. Effects of RA on the temporal relationships between epidermal hyperplasia and dermal biosynthesis of collagen and GAG's in guinea pig skin were determined during a 40-day period. Hyperplasia (thickness) was not constant during this period, as 3 distinct cycles occurred. These cycles were preceded by cycles of collagen biosynthesis rates (^{3}H-proline incorporation into ^{3}H-hydroxyproline) which was preceded by cycles of GAG biosynthesis rates (^{3}H-glucosamine incorporation into papain-resistant macromolecules). After 40 days, dermal blood flow rates (laser doppler photometry were increased 80% in RA-treated compared to control water-treated skin. We conclude that chronic treatment of skin with RA induces marked changes in epidermal and dermal biology. The epidermal hyperplastic response and the dermal fibroblast biosynthetic activities are stimulated, but these responses are then attenuated by unknown regulatory mechanisms.

66. Carbonyl Iron in the Treatment of Hepatic Failure in Erythropoietic Protoporphyria (EPP). V. R. GORDEUK, G. M. BRITTENHAM, H. MUKHTAR, C. W. HAWKINS, AND D. R. BICKERS, Dept. of Medicine, Cleveland Metropolitan Hospital, and Dept. of Dermatology, Case Western Reserve University and Veterans Administration Medical Center, Cleveland, OH.

EPP, an autosomal dominant disorder of heme synthesis, is characterized by deficient ferrochelatase activity, by acute cutaneous photosensitivity and by increased protoporphyrin (PROTO) in erythrocytes, plasma and feces. Iron deficiency anemia, which can further increase erythrocyte PROTO levels, also occurs in some individuals with EPP. Life-threatening hepatotoxicity may occur as a result of retained crystallized PROTO in the hepatocyte. It is known that ferrous iron can insert non-enzymatically into PROTO to form heme. This study evaluated whether iron loading could reduce the excessive body burden of PROTO in a 52 year-old female with EPP, iron deficiency anemia and impending hepatic failure. Due to the appreciable morbidity of conventional iron salts, carbonyl iron, a highly purified (>98%), relatively non-toxic form of elemental iron (particle size $< 5\ \mu$) was employed. The drug was administered orally in daily doses ranging from 400–4000 mg over a 15 week period to a total dose of 90,000 mg. Free erythrocyte PROTO (FEP) was 34,229 μg/dl prior to beginning carbonyl iron (normal < 90 μg/dl) and decreased by >80% to 6430 μg/dl after 15 weeks. The decline in FEP closely correlated with the log of the cumulative dose of carbonyl iron ($r = 0.97$, $p < 0.0001$). Hepatic PROTO decreased similarly, fecal PROTO excretion increased and liver function tests normalized by the end of the treatment period. These studies indicate that patients with EPP and iron deficiency anemia may be particularly susceptible to hepatotoxicity and that carbonyl iron may be useful in managing this potentially fatal complication.

67. Involvement of Receptor Binding and Membrane Permeability in Potentiation of Topical Glucocorticoid. YUTAKA MIZUSHIMA, KEIKO HOSHI, RIE IGARASHI, MAKOTO MURAMATSU*, AND ATSUKO FUJITA*, 1st Dept. of Internal Medicine, St. Marianna University, Kawasaki, Japan, and *Research Center, Taisho Pharmaceutical Co., Ltd., Ohmiya, Japan.

The trigger step in glucocorticid (GC) action is the binding to specific cytoplasmic protein. To bind the receptor, however, GC has to be incorporated into the cells. Therefore, the permeability in the target cell membrane is also has to be given attention to investigate the action of GC. To clarify the involvement of above two things in GC potency, we investigated the binding activity to the receptor and the permeability in the human lymphocytes using hydrocortisone (HC) and HC derivative, HC-17-butyrate 21-propionate (HBP) which has significantly higher potency of anti-inflammatory effects and significantly higher affinity for GC receptor than HC in rats. Similarly to the anti-inflammatory activity of HBP, HBP showed significantly potent inhibitory effect on mitogen induced ^{3}H-tymidine incorporation into the lymphocytes compared with that of HC. The binding activity to the receptor of HBP was higher than that of HC. For the incorporation of GC to the lymphocytes, the amount of ^{3}H-HBP was significantly larger than that of ^{3}H-HC. These results suggest that the permeability of GC may be related to the potency of GC, in addition to its binding activity to the receptor. Furthermore, these characteristics of HBP may be owing to the esterification of OH groups of HC.

68. Effects of Novobiocin on the Replication and Repair of DNA and on Gene Expression in Cultured Human Keratinocytes. V. BOHR AND J. N. MANSBRIDGE, Psoriasis Research Institute, Stanford, CA.

In the course of our studies on the suitability of drugs for the treatment of psoriasis, we have searched for drugs which inhibit DNA replication without damaging DNA or affecting DNA repair. We have also investigated induction of 67 kilodalton (kd) keratin and repression of the ψ-3 antigen to assess their ability to stimulate normal epidermal maturation.

We have found that novobiocin, a topoisomerase II inhibitor, does not provoke a repair response and thus does not appear to damage the DNA. Following irradiation with 20 J/m^2 UVA, cells were incubated in the presence of ^{3}H-thymidine and bromodeoxyuridine with or without novobiocin. The DNA was then fractionated by CsCl density gradient centrifugation. ^{3}H incorporation into the light region of the gradient was taken as a measure of DNA repair and that into the heavier region as replication. Novobiocin, at 0.5 mM, was found to cause a 35% inhibition of DNA repair but a 62% inhibition of the residual semiconservative replication found in the UV-irradiated cells. The drug thus inhibits DNA replication to a greater extent than DNA repair.

Incubation of the cells for 24–48 hours in the presence of increasing concentrations of novobiocin caused a sigmoidal inhibition of ^{35}S-methionine incorporation into acid-soluble material with 50% inhibition at about 0.45 mM. The labelled keratinocytes were fractionated by successive extractions with 1% triton X-100, CASC and 1% SDS/5% 2-mercaptoethanol and the proteins separated by two-dimensional PAGE. Novobiocin at 0.5 mM caused a number of changes in the triton-soluble proteins, including both the disappearance of some species and the appearance of new ones. The pattern of CASC-soluble proteins also changed, notably in reduction in the 58 kd band and the appearance of a band corresponding approximately to 67 kd. Novobiocin at 0.5 mM, which inhibits protein synthesis by approximately 50%, causes considerable modification of gene expression in keratinocytes.

69. Deposition of Human Skin Chymotryptic Proteinase at Dermo-Epidermal Junction. SHIGETOSHI SAYAMA, JOHN K. CHOI, GERALD S. LAZARUS, AND NORMAN M. SCHECHTER, Dept. of Dermatology, University of Pennsylvania, School of Medicine, Philadelphia, PA.

Human skin contains a chymotrypsin-like proteinase which is capable of producing dermo-epidermal separation in the plane of the lamina lucida. The proteinase has been localized by immunofluorescence to human skin mast cells using an antiserum to the purified proteinase. While performing certain control studies to absorb out mast cell fluorescence by pre-incubating antiserum with various concentrations of purified enzyme, it became apparent that free enzyme may bind to the dermo-epidermal junction. To pursue this observation further, we incubated 4 micron human skin sections with purified proteinase prior to treatment with the antiproteinase antibody. A linear band of fluorescence was noticed just under the basal cells, suggesting

the proteinase may have bound to the dermo-epidermal junction. Inhibition of the proteinase by phenylmethanesulfonyl fluoride did not block binding indicating we were not trapping a catalytic intermediate. Pretreating proteinase with antiproteinase antibody prevented binding. Basement membranes are negatively charged structures and an attempt was made to determine whether poly-L-lysine, a polycationic protein could block the binding. 10 mM poly-L-lysine effectively prevented fluorescent deposition indicating the electrostic nature of the interaction. These studies suggest that the human skin chymotrypsin-like proteinase when/if released from mast cells may accumulate along the dermo-epidermal junction. This interaction is significant in view of the effectiveness of the proteinase to degrade the dermo-epidermal junction.

70. Retinoid Effect on Calcium, Phospholipid-Dependent Protein Kinase from Mouse Skin. RAJ KUMAR AND OKSANA HOLIAN, Depts. of Dermatology and Surgery, University of Illinois, Health Sciences Center, Chicago, IL.

Retinol (vitamin A) and its analogs are known regulators of cell differentiation and proliferation. Cell growth and differentiation are also influenced by altered levels of Ca^{++} ions, cyclic AMP and protein kinase activities. A Ca^{++}-activated, phospholipid-dependent protein kinase (C-kinase) activity present in various tissues appears to serve as a receptor for tumor-promoting phorbol esters and to control retinoic acid-induced differentiation of certain embryonal carcinoma cells.

In recent years, some retinoids have been effectively used in the treatment of proliferative skin disorders like psoriasis. This report describes the effect of several retinoids on C-kinase activity in skin of hairless mice.

C-kinase was partially purified from skin of hairless mice by DE-52 ion-exchange chromatography and Sephadex G-200 gel filtration. This enzyme activity was stimulated 15-fold by addition of phosphatidylserine and diolein mixture (PS-DO) in the presence of Ca^{++}. The following compounds were tested for their effects on enzyme activity: β-carotene, retinol, retinal, retinoic acid, etretinate and isotretinoin. Retinoic acid, etretinate and isotretinoin stimulated enzyme activity in the absence of PS-DO, but inhibited PS-DO stimulated activity. The remaining compounds had no significant effect on C-kinase. These results suggest that the active retinoids and phospholipids may compete for the same binding sites on the enzyme.

(Partially supported by a grant from Hoffmann-LaRoche.)

71. Hydrolysis of Hydrocortisone 17-Butyrate 21-Propionate by Cultured Human Keratinocytes. YUKIO KITANO, Dept. of Dermatology, Osaka University School of Medicine, Osaka, Japan.

Hydrocortisone 17-butyrate 21-propionate (HBP) is a synthetic glucocorticoid developed for the topical antiinflammation drug. It is known that esterification at 17 and 21 of glucocorticoid enhances the local action and it is expected that the compound is converted to a physiological glucocorticoid by the metabolism in the skin. After topical application HBP passes through the epidermis and reaches to the dermis where it finds the way to get into the systemic circulation. It necessary to know how is the drug changed in the epidermis. The cultured keratinocytes can be used as a substitute of the epidermis in vivo. The 20 to 30 day-cultures of human keratinocytes were incubated in Eagle's minimum essential medium (MEM) containing steroids, and the medium and cells were analysed by HPLC. At 6 h after the addition of 10^{-5}M HBP, most of HBP was deesterified at the position of 21, and 1519 ng/ml of hydrocortisone 17-butyrate was found in MEM. A small amount of hydrocortisone 21-propionate was formed, but hydrocortisone was not detected. Hydrocortisone was stable in MEM and in the culture of keratinocytes. The intracellular water volume was estimated by measuring the equilibrium uptake of ^{3}H-3-o-methyl-D-glucose, and the intracellular concentration of HBP was calculated. HBP was more than six times concentrated in the cells than in MEM. These results indicated that HBP applied on the skin was taken up by the keratinocytes, hydrolyzed at the position of 21, and reached to the dermis.

72. Kinetics of Epidermal DNA Synthesis in Hairless Mice After Multiple Anthralin Applications. K. YOSHINO AND H. I. MAIBACH, Dept. of Dermatology, University of California, San Francisco, CA.

Anthralin, a potent antipsoriatic drug, produces no systemic side effects, but often induces inflammation. It is believed to combine with DNA, thus inducing cell damage and subsequent inflammation. The effect of multiple doses of anthralin on epidermal DNA synthesis is not known. We have investigated this effect by applying 0.1% anthralin (50 μl of acetone solution) at 0, 2, 4 and 6 days (single to 4 application) to HRS/J hairless mice. From 1 to 12 days, 3 mice (at each point) were sacrificed and 3H-thymidine incorporation in epidermal cells was measured by a modified disc procedure (K. Yoshino et al., Clinical Research. 32: 144A, 1984). After single application, DNA synthesis decreased (up to 90%) at 1 day post application, while it returned to normal level and rebounded (500%) at 4 days later. Then, it returned to normal level at 6 days. DNA synthesis suppression after a second application at 2 days, was not observed; instead there was enhanced stimulation (700%) at 4 days. DNA synthesis, in the groups with 3 and 4 application, revealed the same pattern as in the 2 days application group. These parodoxil kinetics of epidermal DNA synthesis resulting from the 2nd, 3rd and 4th application requires explanation; why does the drug work in psoriasis and is it possible that certain dosage schedules (such as used here) slow lesion resolution and enhance irritation?

73. Comparison of In Vivo and In Vitro Transdermal Drug Absorption and Dermal Drug Metabolism of Vidarabine Using a Unique Isolated Skin Flap Model. S. A. BURTON, Z. J. WOJCIECHOWSKI, U. ROHR, G. G. KRUEGER, AND W. I. HIGUCHI. Depts. of Pharmaceutics and Medicine (Dermatology), University of Utah School of Medicine, Salt Lake City, UT.

Recently, a nude rat sandwich skin flap model has been developed that permits the investigator to sample transdermally absorbed drug before it enters the general systemic circulation where it is subject to extradermal metabolism. It was decided to use this unique model to compare the in vitro and in vivo transdermal absorption and dermal metabolism of vidarabine, an antiviral compound. Vidarabine (ara-A) is deaminated to ara-H in the skin by the enzyme adenosine derminase. The in vitro experiments consisted of measuring the flux of ^{3}H-ara-A and ^{3}H-ara-H across tape-stripped nude rat skin mounted in a diffusion cell at 37°C. Backflux of ^{3}H-ara-H into the donor chamber was also monitored. In the in vivo experiments, a water jacketed half diffusion cell was affixed to tape-stripped sandwich flap skin. ^{3}H-ara-A was deposited in the half diffusion cell and aliquots of flap and systemic blood were periodically taken and analyzed for ^{3}H-ara-A and ^{3}H-ara-H. In vivo ^{3}H-ara-H backflux was also monitored.

Parameter	in vitro (n = 4)	in vivo (n = 2)
• Permeability coefficient (cm/sec)	$2.35 \pm 0.17 \times 10^{-6}$	$2.75 \pm 0.21 \times 10^{-6}$
• Lag time (min)	22.1 ± 1.2	21.0 ± 1.4
• % ara-A dermal metabolism	71.4 ± 1.3	62.6 ± 0.7
• Normalized backflux ara-H	$1.95 \pm 0.21 \times 10^{-5}$	$1.70 \pm 0.14 \times 10^{-5}$

We conclude that in vitro transdermal diffusion and dermal metabolism data can be used to quantitatively predict in vivo transdermal diffusion and dermal metabolism.

1:30 PM–2:15 PM Presidential Ballroom

HERMAN BEERMAN AWARDEE

William Epstein, Presiding

Thomas Waldmann

"T-Cell Receptors: Implications for Diagnosis and Treatment of Cutaneous T-Cell Lymphoma"

2:30 PM–5:30 PM

CONCURRENT SCIENTIFIC SESSIONS

CONCURRENT SESSION 3A IMMUNOCOMPETENT CELLS OF EPIDERMIS

Presidential Ballroom

Sadao Imamura and Robert Tigelaar, Presiding

1. Monoclonal Autoantibodies to Keratins Distinguish Subsets of Human Skin and Thymic Epithelium. K. SINGER, A. ROBERTSON, R. SCEARCE, L. HENSLEY, AND B. HAYNES, Dept. of Medicine, Duke University Medical Center, Durham, NC.

Low titer autoantibodies frequently occur in the sera of normal individuals and include the U-CYT and SC Ab shown by Hintner et al to bind keratin filaments (KF) (JCI 71:1344, JID 82:491). The pathologic consequences of the antibodies are not known. Using spleen cells from unimmunized Balb/C mice, 2 monoclonal antibodies (MoAb) were raised which bind to KF. These MoAb (12F8, 11F1) are both IgM and

have similar tissue distributions, binding stratified epithelia. 12F8 bound to the entire epidermis. In contrast, 11F1 bound strongly to basal keratinocytes and weakly to a subset of suprabasal cells. Within thymus 12F8 bound to all keratinized epithelium, whereas 11F1 bound only to endocrine epithelium (subcapsular cortex and medulla) and not to nonendocrine keratinized cortical epithelium. Double fluorescent assays confirmed that within thymus 11F1+ cells represented a subset of keratinized thymic epithelial cells. Subpopulations of both cultured human skin epithelial cells (HSEC) and cultured human thymic epithelial cells (HTEC) were positive for 11F1 (50–95%) or 12F8 (50–91%). Immunoblots using detergent insoluble keratin preparations from cultured cells demonstrated that 12F8 bound to keratin bands including 48 and 50 kd in both HSEC and HTEC. 11F1 recognized the 48 and 50 kd as well as 56 and 58 kd keratins. These immunoblot patterns differed from those of known anti-keratin antibodies AE1, AE2 and AE3. Thus, normal mouse splenic B cells produce antibodies to keratin filaments. Autoantibodies of similar specificity may account for antibodies with multiple organ reactivity reported in autoimmune disorders. Because of their unique specificities, antibodies 12F8 and 11F1 will be of great value in studying the function of subsets of skin and thymic epithelium.

2. The Role of Epidermal Cells in the Induction of Delayed Type Hypersensitivity (DTH) to Allo-antigens. KUNIHIKO TAMAKI AND MASUTAKA FURUE, Dept. of Dermatology, University of Tokyo, Tokyo, Japan.

Allogeneic skin grafts can induce DTH both to the gene products coded by the major histocompatibility complex (MHC) and to non-MHC as assessed by foot pad swelling (FPS). Recent studies suggest that skin graft rejection and DTH are different manifestations of the same mechanism. To investigate the alloantigenicity of epidermal cells (EC), we tried to induce DTH using allogeneic EC. EC suspensions were obtained from C3H mice, and 1×10 EC were injected subcutaneously (sc) into BALB/c mice. Seven days later, 4×10^6 C3H spleen cells (SC) were injected intradermally into left hind foot pad and FPS was measured 24 hrs later. This could induce marked FPS. In attempts to transfer DTH, SC were obtained 7 days after sensitization and were injected intravenously into syngeneic mice. This also induced DTH. When these SC were treated with anti-theta antisera and complement, DTH was no longer detected. Further experiments have shown that these effector T cells were Lyt-1^+, Lyt-2^-. Experiments, examining whether both H-2 and non-H-2 antigens contribute to the induction of DTH, were performed using congenic strains of mice. Although either H-2 or non-H-2 disparities induced marked FPS, Ia disparities induced weak but significant DTH reaction. Thus, the results established the precise conditions to induce allo-DTH using EC, and have revealed the contribution of the gene products coded by MHC and non-MHC.

3. A Monoclonal Antibody Identifies a Membrane-Associated Antigen Which Is Expressed at Sites of Keratinocyte to Keratinocyte Contact. T. I. DORAN, P. R. BERGSTRESSER, AND D. R. HIGHLEY, Mary Kay Cosmetics and Dept. of Dermatology, UTHSCD/Southwestern Medical School, Dallas, TX.

Monoclonal antibodies have been used to study the localization of cellular components and their distribution in different animal species. To investigate keratinocyte structure and function, monoclonal antibodies were generated by the fusion of SP 2/0 myeloma cells with spleen cells from Balb/c mice immunized with the Tris-insoluble proteins from adult epidermis. Using indirect immunofluorescence, monoclonal antibody AG9 stained the membranes of adult and foreskin human epidermal cells in a particulate fashion. By contrast, regions of basal cells in direct contact with basement membrane were conspicuously negative. This antibody also stained the membranes of epithelial cells from rabbit skin and esophagus in a similar fashion. Epithelial cells from adult human skin, rabbit esophagus and skin were grown in the presence of lethally irradiated 3T3 fibroblasts. The presence of epithelial colonies in tissue culture was confirmed with anti-keratin antibody BG2, which stained only basal cells in human and rabbit epithelia. With AG9, human epidermal cells in tissue culture also displayed a particulate membrane staining which was apparent only at the point of contact between such cells. Additionally, cultured rabbit epidermal and esophageal epithelial cells were stained in a similar fashion. 3T3 fibroblasts, used as feeder cells for growth of epithelial cells, showed no such staining. The association of this antibody staining with epithelial cell-cell contact suggests that the antigen which it identifies plays a role in cell attachment or communication.

4. Recombinant Gamma Interferon Induces HLA-DR Antigen Expression on Trichilemmoma, Gastric Adenocarcinoma Cell Lines and Cultured Human Keratinocytes. KOICHIRO KAMEYAMA, HIKARU ETO, SHIN-ICHIRO TAKEZAKI, AND TAMOTSU KANZAKI, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Although, recombinant gamma interferon (r-IFN-gamma) has been shown to induce HLA-DR biosynthesis and expression on cultured normal human keratinocytes and human malignant melanoma cell lines, there is no report concerning the effect of r-IFN-gamma on cultured epithelial cell lines. In the present study, we investigated the effect of r-IFN-gamma in induction of HLA-DR expression on human trichilemmoma, gastric adenocarcinoma, eccrine carcinoma cell lines and cultured keratinocytes. Various epithelial cell lines were plated in 35 mm tissue culture dishes at 10^6 cells/2ml Dulbecco's modified Eagles medium (DMEM) with 10% fetal calf serum (FCS) and r-IFN-gamma (500 units/ml). Normal keratinocytes were prepared from surgical specimens, Langerhans cells were depleted by panning techniques, and viable cells (5×10^6) were seeded in a 24-well multidish in 1ml DMEM with 10% FCS. The cells were incubated at 37°C with 5% CO2 and r-IFN-gamma (500 units/ml) was added 1 week after the cells were seeded. Three days after adding r-IFN-gamma, these cells were analysed by fluorescence-activated cell sorter. It was found that HLA-DR was expressed in one out of one cultured keratinocytes, 3 out of 3 trichilemmoma and one out of one gastric adenocarcinoma cell lines. Only one eccrine carcinoma cell line failed to express HLA-DR after r-IFN-gamma treatment. In this study, we found, for the first time, that r-IFN-gamma induce HLA-DR expression on established epithelial tumor cell lines. Since HLA-DR expression has been shown to be important for immune response, these findings suggest that r-IFN-gamma plays an important role in tumor immunology.

5. Immunoprecipitation of HLA-DR Antigens from Gamma Interferon-Stimulated Cultured Human Keratinocytes. NORMAN WIKNER, MARK KISSINGER, DAVID NORRIS, J. CLARK HUFF, AND WILLIAM WESTON, Dept. of Dermatology, University of Colorado School of Medicine, Denver, CO.

Expression of HLA-DR antigens in the cells of the normal human epidermis is confined to the Langerhans cells and indeterminant cells. Keratinocytes, however, may stain for such Class II antigens in certain skin diseases characterized by mononuclear cell infiltrates, such as lichen planus, erythema multiforme, and graft vs. host disease. Previous studies identified the keratinocyte as the source of the HLA-DR antigens seen in murine graft vs. host disease. *In vitro* experiments have shown that gamma (immune) interferon stimulates HLA-DR expression. The purpose of this study is to demonstrate by immunoprecipitation techniques that gamma interferon-stimulated keratinocytes synthesize the peptides that constitute such Class II antigens.

Second passage human keratinocytes isolated from neonatal foreskins were grown in serum-free, defined medium without a feeder layer. The cells in these cultures were 100% keratinocytes, as demonstrated by positive immunofluorescence (IF) staining for keratin and negative IF staining for HLA-DR and OKT6. Keratinocyte cultures were stimulated for two days with recombinant human gamma interferon (0–50 units) and pulse labeled with 35S methionine. The cells were lysed and immunoprecipitation was performed with a monoclonal antibody to human HLA-DR (L243 IgG2a) and staphylococcal protein A. Evaluation of the immunoprecipitated proteins by SDS-polyacrylamide gel electrophoresis and autoradiography demonstrated labeled proteins with molecular weights corresponding to the alpha and beta chains of human HLA-DR. The amount of HLA-DR synthesis was directly related to the dose of gamma interferon used for stimulation. This study confirms by immunoprecipitation techniques that pure keratinocyte cultures can synthesize HLA-DR when stimulated by gamma interferon.

6. Bone Marrow-Derived Cells in Normal Human Epidermis: A Search for Thy-1^+ Cells, Lymphocytes, and Macrophages. K. D. COOPER, S. M. BREATHNACH, S. W. CAUGHMAN, A. PALINI, M. WAXDAL, AND S. I. KATZ, Dermatology Branch, NIH, Bethesda, MD.

It has been proposed that the epidermis may provide an inductive microenvironment for specific populations of recirculating epidermotropic lymphocytes. As dendritic, Thy-1^+, bone marrow-derived epidermal cells (EC), distinct from Langerhans cells (LC), have been identified in mice, we sought to identify their potential human analogue, as

well as other bone marrow-derived cells, in epidermis using anti-leukocyte reagents. We used a double fluorescent staining technique with anti HLe-1, an antibody which identifies all bone marrow-derived human leukocytes, and anti HLA-Dr, which identifies epidermal LC. The vast majority of HLe-1$^+$ EC were HLA-Dr$^+$ LC; these were present at a mean density of 608 cells/mm^2 in epidermal sheets and 1.4% of viable EC in suspension, as determined by flow cytometry. The non-dendritic morphology and low frequency of HLe-1$^+$ Dr$^-$ EC in sheets (mean: 3.7 cells/mm^2) and suspensions (mean: 0.06% of EC) precluded their representing a strict human analogue of the murine Thy-1$^+$ EC, since murine Thy-1$^+$ EC are dendritic and are present in a similar density to LC. Preparations of the minor HLe-1$^+$ Dr$^-$ EC population purified by flow cytometry or panning and examined ultrastructurally were not enriched for any type of leukocyte population. Further studies did not detect significant numbers of human Thy-1 antigen bearing cells, T or B cells, macrophages (other than LC) or natural killer cells in tissue sections, epidermal sheets and gradient-enriched EC suspensions. Thus, using currently available markers and sorting technology, we have been unable to identify a human analogue of the murine Thy-1$^+$ dendritic epidermal cell. In addition, these findings indicate that T cells and other leukocytes, whether resident or epidermotropic, occur only very rarely in normal human epidermis.

7. Culture and Characterization of Murine Thy-1$^+$ Epidermal Cells. S. W. Caughman, S. M. Breathnach, and S. I. Katz, Dermatology Branch, NIH, Bethesda, MD.

Elucidation of the biologic role of the murine dendritic Thy-1$^+$ epidermal cell (EC) would be greatly facilitated by its propagation *in vitro*; thus our purpose was to obtain purified populations of Thy-1$^+$ EC through culture techniques. Since Thy-1$^+$ EC are bone marrow-derived, we attempted to grow this cell selectively from density gradient enriched EC suspensions (FH-EC) using conditions known to promote mitogenesis of marrow-derived subsets. FH-EC prepared from truncal skin of C3H mice were cultured at 37°C in 5% CO_2 in complete medium (CM) of EHAA with 10% fetal calf serum, L-glutamine, antibiotics, and 2-mercaptoethanol at 10^6 FH-EC/well in 24-well culture plates. In certain experiments, CM was supplemented with one or more of the following: conconavalin A (Con A), interleukin (IL-) 1/ETAF, IL-2, IL-3, γ interferon, indomethacin 1 μg/ml (IM) and anti-Thy-1.2 antibody. Media were changed every 2–3 d. Fresh FH-EC contained 7–20% Thy-1$^+$ cells which were also asialo GM$_1^+$, Ly 5$^+$, vimentin$^+$, and negative for other T-, B-, and macrophage cell markers. Phenotypic studies in ten separate cultures revealed 3- to 10- fold expansion of Thy-1$^+$ cells after 21 ± 4 d in CM with Con A 0.5 μg/ml and IM, and 70 to 95% of viable cells were Thy-1$^+$. In certain cultures, expanded Thy-1$^+$ cells were all phenotypically identical to fresh Thy-1$^+$ EC, while other cultures resulted in Thy-1$^+$, asialo GM$_1^-$, L3T4$^+$ cells. Selective cytolytic pretreatment of fresh EC preparations failed to prevent the appearance of Thy-1$^+$, L3T4$^+$ cells after 21 d. These studies indicate that Con A stimulation of murine EC suspensions in culture can facilitate the proliferation of either Thy-1$^+$, asialo GM$_1^-$, L3T4$^+$ (T-helper phenotype), or Thy-1$^+$, asialo GM$_1^+$, L3T4$^-$ (natural killer phenotype) cells and may thus be useful in characterizing the functional role of the murine dendritic Thy-1$^+$ EC.

8. Heterogeneity of Murine Thy-1$^+$ Epidermal Cells. R. L. Cohen, M. Ostrega, B. Coyne, M. Alves, and D. A. Chambers, Health Science Center, University of Illinois at Chicago, Chicago, IL.

Previous investigations in this and other laboratories have revealed the presence of unique subpopulations of cells in the epidermis which bear the cell-surface protein, Thy-1. The present studies were designed to determine if Thy-1+ epidermal cells are a homogeneous or heterogeneous population. Since vimentin is the intermediate filament protein of cells of mesenchymal origin, including bone-marrow derived cells, and keratin is that characteristic of keratinocytes, epidermal sheets and cell suspensions derived from BALB/c mouse ear and flank skin were double stained for immunofluorescence microscopy utilizing monoclonal antibodies directed against Thy-1 (Becton Dickinson, Mtn. View, CA), vimentin (Lab Systems, Chicago) and keratin (provided by Dr. T-T Sun, NYU Med. Ctr., New York). The following classes of epidermal cells were observed: Thy-1 + /Vim+; Thy-1 + /Vim$^-$; Thy-1 + /Ker+; Thy-1+/Ker$^-$; Thy-1$^-$/Vim+; Thy-1$^-$/Ker+. Epidermal cell cultures analyzed analogously for Thy-1 and keratin contained Thy-1 + /Ker+, Thy-1 + /Ker$^-$ and Thy-1$^-$/Ker+ cells. To determine if an association exists between Thy-1+ epidermal cells and NK cells, which bear cell-surface asialoGM$_1$, parallel experiments were performed utilizing a polyclonal anti-asialoGM$_1$ antibody (Wako Chemicals, Dallas) on NK cell-deficient Beige mice and C57BL wild type mice. These experiments reveal that the mutant Beige mouse contains Thy-1+ cells and no asialoGM$_1$+ cells, whereas the normal C57BL mouse has Thy-1 + /asialoGM$_1$+ and Thy-1 + /asialoGM$_1^-$ cells. These experiments reveal that Thy-1+ epidermal cells are heterogeneous and include, but are not exclusively, keratinocytes (Thy-1 + /Ker+), bone-marrow derived (Thy-1 + /Vim+) and NK-like cells (Thy-1 + /asialoGM$_1^+$).

9. Induction of Birbeck Granule-like Structures in Murine T-Lymphocytes and Dendritic Thy-1-positive Epidermal Cells by a T Cell-Activating Anti-Thy-1 Monoclonal Antibody. Nikolaus Romani, Georg Stingl, Erwin Tschachler, Kurt C. Gunter, Ethan M. Shevach, and Gerold Schuler, Dept. of Dermatology, University of Innsbruck, Innsbruck, Austria, Dept. of Dermatology I, University of Vienna, Austria, LI, NIAID, NIH, Bethesda, MD, and Rockefeller University, New York, NY.

Birbeck granule-like structures (BGLS) are induced in dendritic Thy-1-positive epidermal cells (dThy-1$^+$EC) by the binding of G7, a T cell-activating monoclonal anti-Thy-1 antibody, but not by conventional anti-Thy-1 reagents. Reasoning that G7-induced BGLS formation may be a more generalized phenomenon we studied the morphological events following exposure of peripheral T cells to monoclonal antibody G7. Suspensions of C3H/He and Balb/c peripheral T cells, T cell hybridomas (AF3G7 and P-4-3), thymocytes and EC were incubated with G7 under conditions optimal for BGLS induction (40 min/0°C - 30 min/37°C). Electronmicroscopic examination of G7-treated T cells, T hybridoma cells and thymocytes revealed the presence of BGLS in at least 30% of the cells. The BGLS were primarily observed at cell-to-cell contacts and at sites of surface membrane invaginations. Cross-linking with a second antibody was not necessary for the appearance of BGLS. No BGLS were observed after incubation with a non T cell-activating rat monoclonal anti-Thy-1 reagent (B5-3). Further studies with dThy-1$^+$EC showed that G7-induced BGLS formation was not inhibited by preincubation with conventional monoclonal anti-Thy-1 antibodies. The presence of Fab fragments of G7 sufficed neither for BGLS induction nor for T cell activation. Thus, monoclonal antibody G7 seems to recognize and thereby crosslink particular epitopes of the Thy-1 molecules on T cells as well as on dThy-1$^+$EC. This phenomenon may not only be a morphological correlate of an early event in T cell activation but represents a more generalized structural consequence of receptor-ligand interactions than previously thought.

10. Ontogenetic Expression of HLA-DR and OKT-6 Determinants by Human Embryonic and Fetal Langerhans Cells. Carolyn A. Foster and Karen A. Holbrook, Dept. of Biological Structure, University of Washington, School of Medicine, Seattle, WA.

The presence and density of Langerhans cells (LCs) in human fetal skin were investigated, and the developmental expression of HLA-DR and OKT-6 antigens was quantitated. Skin from the lower limb was excised from 35 embryos and fetuses, ranging in age from 43–170 d, and incubated in EDTA to separate epidermis from dermis. The epidermal sheets were fixed, immunolabeled for HLA-DR and OKT-6, and prepared for light and electron microscopy; some were double-labeled for ATPase activity and either DR or T6 antigen. The number of DR$^+$ and T6$^+$ cells/mm^2 of epidermis was determined on 50 fields/specimen at 40 X, using a calibrated ocular grid. Labeled cells/mm^3 were calculated from volume measurements using digitized skin sections.

LCs were present in the epidermis by at least 43 d but were smaller and less dendritic in embryonic than in fetal epidermis. Some were DR$^-$, ca. 80/mm^2 (300/mm^3) were DR$^+$, and all were ATPase$^+$, T6$^-$. In the second half of the 1st trimester the number of DR$^+$ cells ranged from 20–260/mm^2 (60–7000/mm^3), but the data suggested a cyclic pattern rather than a constant increase in LCs density. By the 2nd trimester the number of DR$^+$ cells plateaued at 100/mm^2 (2000/mm^3), then gradually increased to 250/mm^2 (4500/mm^3) by the 3rd trimester. At the transition from the embryonic to the fetal period (ca. 60 d), LCs began to express T6. In contrast to DR, T6 expression was only 1–25 cells/mm^2 until ca. 85 d when the number of T6$^+$ cells rapidly increased, resulting in equal density (100/mm^2) of T6$^+$ and DR$^+$ cells. This apparent pattern of coexpression, and thus similar density of labeled cells, continued throughout the 2nd trimester.

11. T4/Leu-3 Expression on Epidermal Langerhans Cells—A Dynamic Event. VERONIKA GROH, MASAHIRO TANI, ANDREA HARRER, KLAUS WOLFF, AND GEORG STINGL, Dept. of Dermatology I, University of Vienna, Vienna, Austria.

Recent evidence exists that the T4/Leu-3 antigen can be detected on mononuclear phagocytes and epidermal Langerhans cells (LC). When searching for T4/Leu-3 expression in tissue sections of inflammatory and neoplastic skin disorders we observed great fluctuations in the intensity of Leu-3 labeling of epidermal dendritic cells. Reasoning that the extent of T4/Leu-3 expression by these cells might be an induceable event, we compared the Leu-3 LC staining pattern in clinically normal appearing skin (CNAS) with that occurring in several skin disorders. In cryostat sections of 19 biopsies taken from different body regions of CNAS Leu-3$^+$ dendritic epidermal cells were visualized by a four-step immunoperoxidase technique at the threshold of detectability. Using both OKT6/Leu-3 double-labeling and immunelectronmicroscopy, these cells qualified as LC. Studies on epidermal sheets of CNAS revealed, however, that the density of Leu-3$^+$ LC was considerably less than that of T6 bearing LC (808 ± 38/mm^2 vs. 388 ± 29/mm^2). In sharp contrast to CNAS, strong and prominent anti-Leu-3 LC labeling was almost invariably encountered in biopsy specimens from patients with cutaneous T cell lymphoma and various inflammatory skin disorders. The extent of Leu-3 LC staining in proliferative disorders of resident skin cells (nevus cell nevus, dermatofibroma) was comparable to that seen in CNAS.

Our results strongly suggest that the degree of Leu-3 expression on epidermal LC is regulated by signals from inflammatory cells. The induction of putative class II alloantigen receptors (T4/Leu-3) on class II alloantigen-bearing LC may represent an important regulation mechanism of antigen-presenting cell function.

12. Evidence That Human Peripheral Blood Dendritic Cells Are Not Langerhans Cell Precursors. ROBIN SLOVAK, BRIAN BERMAN, AND MATTHEW DUNCAN, Depts. of Dermatology, University of California at Davis and Veterans Administration Medical Center, Martinez, CA.

Langerhans cells (LC) are epidermal cells of hematopoietic origin capable of antigen presentation and accessory cell functions. Dendritic cells (DC) are accessory cells found in lymphoreticular tissue and peripheral blood. To investigate whether DC are circulating progenitors of LC, we initially isolated and enriched DC from human peripheral blood mononuclear cells via their distinctive adherent properties followed by discountinuous bovine serum albumin density gradient centrifugation. These isolated low density DC (1.05/1.08 interface) displayed limited adherence to plastic, spread on poly-L-lysine coated coverslips, bore DR antigens, stimulated mixed leukocyte reactions and lacked Fc receptors and T6 antigens. DC were maintained in tissue culture and exposed for 2–6 days to alpha interferon, beta interferon, gamma interferon, thymopoietin, 1,25 dihydroxycalciferol, granulocyte-monocyte colony stimulating factor, human epidermal sheets, keratinocyte monolayers and their culture medium. T6 antigens were not detected by immunofluorescent microscopy on DC following exposure to these inducing factors and environments. Whereas DC are related to antigen presenting cells in other tissue, our data suggest that these peripheral blood DC are not precursors of epidermal LC, as determined by T6 antigen expression.

13. Attachment of HLA-DR Positive Dendritic Cells to Colonies of Human Keratinocytes *In Vitro*. ROBERT B. HOPP, R. MARK KISSINGER, DAVID A. NORRIS, WILLIAM L. WESTON, AND J. CLARK HUFF, Dept. of Dermatology, University of Colorado School of Medicine, Denver, CO.

Langerhans cells (LC), a specialized intraepithelial subpopulation of human dendritic cells (DC), are strongly HLA-DR positive, may function as accessory cells in certain immune responses, and are potent stimulators of mixed leukocyte reactions. Because DC are present in the peripheral blood, constituting less than 1% of peripheral blood mononuclear cells (PBMC), and because, in animal studies, LC may be derived from bone marrow precursors, it is reasonable that LC might be derived from circulating DC or their precursors. The purpose of this study is to generate and to study mixed cultures of keratinocytes and DC derived from PBMC.

Human keratinocytes (HK) were isolated from neonatal foreskins and grown in serum-free, defined medium. In second passage cultures, 100% of the cells stained for keratin, and expression of HLA-DR and OKT6 was absent. Second passage HK were grown for 7 days in culture chambers, and PBMC populations were added along with fresh medium containing 10% fetal calf serum and allowed to settle over keratinocyte colonies. After 0–5 days of additional culture, the slides were washed, fixed with acetone, and stained by an indirect immunofluorescence (IF) technique for HLA-DR, with use of a monoclonal antibody. After addition of either crude PBMC or Percoll-separated monocytes populations, strongly HLA-DR positive cells with dendritic morphology were found attached to keratinocyte colonies but not to "bare" areas of the glass slide. This culture system with HK and associated HLA-DR positive DC may provide an *in vitro* model for study of the interaction of DC with the epidermis and the acquisition of LC characteristics.

14. Reactivity of Epidermal Langerhans Cells with Monoclonal Antibodies Directed Against the Murine IL-2 Receptor. GEORG STEINER, ERWIN TSCHACHLER, MASAHIRO TANI, THOMAS R. MALEK, ETHAN SHEVACH, AND GEORG STINGL, Dept. of Dermatology I, University of Vienna, Vienna, Austria, and LI, NIAID, NIH, Bethesda, MD.

We have recently produced two rat monoclonal antibodies, designated 3C7 and 7D4 which detect two distinct functional regions of the murine IL-2 receptor. When studying the emergence kinetics of IL-2 receptors in epidermal cell (EC)-lymphocyte cultures by using 7D4 and 3C7 antibodies in an indirect immunofluorescence (IIF) assay, we regularly encountered a distinctive membrane fluorescence not only on lymphocytes, but also on a subpopulation of dendritic cells. Reasoning that these 7D4/3C7-reactive dendritic cells might represent a subpopulation of epidermal dendritic cells, we studied single mouse EC cultures for the presence of 7D4/3C7-reactive cells using IIF techniques. Whereas 7D4/3C7 reactivity was never detected on freshly isolated EC, a small percentage of 7D4/3C7$^+$ cells was encountered after 24–48 h of culture. These cells exhibited a dendritic shape, expressed Ia antigens, lacked Thy-1 antigens, and displayed the ultrastructural features of Langerhans cells (LC) with the noteable exception of Birbeck granules. While, after 24 h only 20% of Ia$^+$EC were 7D4/3C7$^+$, virtually all Ia$^+$EC displayed 7D4/3C7 binding after 7 days of culture.

Our results demonstrate that LC in culture are the only EC to react with monoclonal antibodies against functional regions of the murine IL-2 receptor. Preliminary data showing that recombinant human IL-2 interferes with 3C7 binding to LC further suggest that the 7D4/3C7 binding site on LC is indeed the IL-2 receptor. These results may bear important implications for the regulation of growth, differentiation and immunological functions of LC.

15. Modulation of Epidermal Langerhans Cell (LC): Alterations in Contact Hypersensitivity Reactions to Dinitrofluorobenzene (DNFB) Following Topical Application of Hydroquinone and Arachidonic Acid. L. A. RHEINS AND J. J. NORDLUND, Dept. of Dermatology, University of Cincinnati College of Medicine, Cincinnati, OH.

Topical application of monobenzyl ether of hydroquinone (MBEH) or arachidonic acid (AA) alters the number and morphology of Ia+ LC. To investigate whether higher or lower populations of LC are associated with changes in immune reactivity (IR), we performed these studies. DBA/2 mice were treated with 20% MBEH in ethanol applied topically for seven days either to the ears or to shaved dorsal back skin. Controls were treated with diluent. On day 8, ATPase and Ia+ LC were quantified. Mice treated with MBEH had 57% and 50% more ATPase or Ia+ cells per/mm^2 respectively compared to controls. MBEH treated mice were contact sensitized on the back with DNFB and challenged on the ear on day 15. Ear swelling was measured before and 24 hours post-challenge. Animals sensitized on MBEH treated back or mice sensitized on untreated back and challenged on MBEH treated ears exhibited a two-fold greater increase in ear swelling than controls. AA (2%) applied to the back or ears caused a 43% and 57% decrease in ATPase and Ia+ LC respectively versus controls. Contact sensitivity was suppressed by 43% in mice sensitized on AA treated back or challenged on AA treated ears compared to controls. We have identified a method to increase or decrease identifiable Ia+ cells in murine skin. Skin with more Ia+ cells reacts on sensitization or challenge more vigorously than normal skin. Skin with fewer identifiable LC exhibits, on sensitization or challenge, suppressed IR. The sensitivity of cutaneous IR apparently can be modulated up or down. Whether this is due to a local or systemic mechanism is currently being investigated.

CONCURRENT SESSION 3B: CELL BIOLOGY

Congressional

Yasumasa Ishibashi and Robert Lavker, Presiding

1. The Regulatory Role of Vasoactive Intestinal Peptide (VIP) on Eccrine Sweating. K. SATO AND F. SATO, Marshall Dermatol-

ogy Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

Abundance of VIP nerves around the eccrine sweat gland is already known. We have now attempted to elucidate the role VIP plays in regulation of cholinergic and adrenergic sweating as well as the role of VIP itself as a stimulant of sweat secretion. Single sweat glands teased out from biopsy skin specimens of the monkey palm were cannulated and bathed in Krebs' Ringer buffer (KRB) (pH 7.4) at 37°C. Addition of various doses of VIP to the bath evoked sweat secretion within a minute, although the sweat rate slowly tapered with time. Thus, at 60 minutes the sweat rate fell to 50 to 80% of the peak level. The dose response curve yielded a saturation curve with Km for VIP of about 10^{-8}, M approaching the maximal sweat rate at 5×10^{-8} M to 10^{-7} M. The VIP-induced maximal sweat rate was 35% the maximal cholinergic sweat rate, was not further enhanced by theophylline (10^{-3} M), forskolin (5×10^{-6} M) or isoproterenol (5×10^{-5} M), and was not inhibited by atropine or propranolol. There was synergism between the submaximal concentrations of VIP and methacholine (MCH) on the sweat rate. Unlike cholinergic sweating, VIP-induced sweat secretion is relatively insensitive to Ca^{2+} removal from the bath. Cyclic AMP accumulation in the gland during VIP stimultion was 50% higher than that during isoproterenol (ISO) stimulation and followed the time-course and dose response of VIP-induced sweat secretion. As in ISO, VIP-induced tissue cAMP accumulation was augmented 10 fold by 5×10^{-5} M MCH. The VIP also resembled ISO in its effect on membrane and epithelial potential difference. Thus, although VIP simulates all the characteristics of ISO, it requires two orders of magnitude less concentration than ISO and is therefore potentially more important than ISO in regulation of sweat gland function.

2. Origin of Cystein Proteases in Human Eccrine Sweat. H. YOKOZEKI, N. HORIE, AND K. SATO, Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

Relatively little is known about cystein proteases in human skin. Although human eccrine sweat contains a variety of proteases and proteinases, cystein proteases (CP) are by far the most prominent. CP in sweat, skin eluates, or sweat gland extracts was assayed by incubating an aliquot of samples with a synthetic substrate Leu-N Nap (LNA) in Tris buffered saline (TBS) at the optimal pH of 8.0 at 37°C for 3 hours with or without an SH-agent, dithiothreitol (DTT) and the released chromogen naphthylamine determined spectrophotometrically. We have observed that CP is 10 fold higher in crude (scraped) sweat (SS) than in the least contaminated thermally induced sweat collected in an aerobic plastic bag on vaseline-coated skin (CS), yet the latter activity is still higher than any other proteases activity as determined with various Kabi-S series substrates. Since in vivo elution of CP with TBS for one hour inside the anaerobic plastic bag over the vaseline-coated skin at 25°C does not yield CP activity, CP activity in CS is either of glandular or epidermal origin (but at the level of intraepidermal sweat duct unit) or both. In fact, strong CP activity was noted in eluates of the lower layer of tape-stripped stratum corneum and in in vivo eluates of stripped skin, also in TBS extracts of methacholine-stimulated isolated human sweat glands and in the incubation medium bathing the sweat glands. Sephadex G100 chromatography of SS (CP of mainly epidermal origin) showed a prominent peak at 130K dalton, whereas that of CS (CP of both glandular and epidermal sweat ductal origin) showed a small peak at 130K and a tall peak at 32K. Interestingly, CP is inhibited by 1mM anthralin. Although the physiological function of CP is unknown, both epidermis and sweat glands appear to produce distinct CP's.

3. Permeabilization of Cultured Cells Using Lysolecithin: Application to Flow Cytometric Analysis of Intracytoplasmic Antigens. SHIN-ICHIRO TAKEZAKI, HIKARU ETO, KOICHIRO KAMEYAMA, AND SHIGEO NISHIYAMA, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Lysolecithin (lysophosphatidylcholine) is a unique phospholipid which can permeabilize cell membrane of many types of cells. At a low concentration (around 10 μg/ml), lysolecithin treated cells remain viable, although they are permeabilized and unable to exclude trypan blue. In this study we investigated applicability of this technique to flow cytometric analysis of intracytoplasmic antigens. Cultured human fibroblasts and adeno carcinoma cells were treated with various concentrations of lysolecithin solution (10–100 μg/ml) for two minutes at 4°C. Cells were then incubated with monoclonal antibody which recognizes common intermediate filament antigen (EKH1), and FITC-conjugated anti mouse Ig. By flow cytometric analysis using FACS420, intermediate filament antigen was successfully detected in both types of cells (keratin in adeno ca., vimentin in fibroblast). No increase of background fluorescence was observed. Optimal conditions of lysolecithin treatment differ from cell to cell. In the case of adeno ca. cells, treatment with 10 μg/ml of lysolecithin brought significant but partial permeabilization (84% of cells became positive with EKH1). At higher concentration (50 μg/ml), 95% of treated cells were positive. On the other hand, fibroblasts were more fragile against lysolecithin treatment. Cells were easily lysed and destroyed.

Flow cytometry is a powerful technology in the field of cell biology. However its use has been practically limited to membranous antigens. Permeabilization of cells using lysolecithin will apparently extend the applicability of flow cytometry, and also enable many other experiments to bring non-penetrating molecules into living cells.

4. Lamellar Bodies from Neonatal Mouse Epidermis: Functional Implications of Lipid and Enzyme Content. S. GRAYSON, A. D. JOHNSON-WINEGAR, B. U. WINTROUB, E. H. EPSTEIN, JR., R. R. ISSEROFF, AND P. M. ELIAS, Dermatology Service, VAMC, San Francisco, Dermatology Unit, San Francisco General Hospital, San Francisco, and Depts. of Dermatology, University of California Schools of Medicine, San Francisco and Davis, CA.

Partially-purified fractions of lamellar bodies (LB) have now been isolated from fetal rat and neonatal mouse epidermis. Recent studies by Freinkel and her collaborators suggest that fetal rat LB are enriched in sphingolipids and acid phosphatase. We have compared the content of lipids and certain hydrolases and proteases in homogenates (H) vs. LB-enriched fractions obtained from neonatal mice (Science 221:962, 1983). We found that mouse LB are enriched 2–10 fold over H in acid phosphatase, as well as 2–10 fold in cathepsin B-like and carboyxpeptidase activities, and in acid lipase, phospholipase A2, and sphingomyelinase. The LB fraction was not enriched, however, in the typical lysosomal enzymes, β-glucuronidase and aryl sulfatase A + B, nor in the membrane-associated, non-lysosomal sulfatase, steroid sulfatase, or plasminogen activator, a non-lysosomal protease. Moreover, we found that LB were enriched over H in phospholipids, free sterols, and glycosphingolipids, but not in other neutral lipids, ceramides, or cholesterol sulfate. Taken together, these data support a role for LB in the delivery of lipid precursors to intercellular domains, where they would be metabolized further (in part by LB-associated enzymes) to intercellular products that regulate permeability barrier formation and desquamation. Moreover, the absence of certain specific lysosomal enzymes, and the presence of others, suggests that the LB is not a typical lysosome. The presence of abundant acid phosphatase and proteases in LB suggest a role for this organelle not only in barrier function, but also in desquamation.

5. Synthesis of Arachidonic Acid from Exogenous Linoleic Acid by Cultured Mouse Keratinocytes. R. R. ISSEROFF, D. T. MARTINEZ, R. S. CHAPKIN, AND V. A. ZIBOH, Dept. of Dermatology, School of Medicine, University of California, Davis, CA.

We have previously demonstrated that levels of linoleic acid (18:–12n6) rapidly decrease when mouse keratinocytes are cultivated in FCS-containing medium: a four-fold decrease was observed after 5 days of cultivation. In contrast, levels of arachidonic acid (20:4n6) remain remarkably unchanged during cultivation. It was not known whether the 20:4n6 was biosynthesized by the cells or incorporated from serum fatty acids in the medium. We therefore explored the fate of exogenously supplied 18:2n6 to determine whether cultivated keratinocytes could convert this precursor to 26:4n6. Confluent mouse keratinocyte cultures were incubated for 16 hours in serum-free medium containing ^{14}C-18:2n6. The radiolabelled medium was then removed and replaced with FCS containing medium. At 18 and 64 hours after labelling the lipids of the cultivated cells were extracted and methyl esters of total lipid fatty acids (FAMEs) prepared. The resultant FAMEs were analyzed by three different techniques: thin layer chromatography on silver nitrate-impregnated silica plates, reverse phase HPLC, and reverse phase HPLC of p-bromophenacyl ester derivatives. All three methodologies demonstrated the time-dependent conversion of 18:2n6 to 18:3n6, 20:3n6 and 20:4n6. At 64 hours, 2–3% of 18:2n6 had been converted to 18:3n6; 8–14% to 20:3n6, and 12–18% to 20:4n6. These studies demonstrate that Δ5 and Δ6 desaturases as well as elongase activities are present within cultivated keratinocytes, and that exogenously supplied 18:2n6 can be converted *in vitro* to 20:4n6. The inconsistency between this *in vitro* data and previous classical *in vivo* enzymatic studies is unclear and requires further investigation.

6. Developmental Changes In Human Fetal Skin Lipids. MARY L. WILLIAMS, MARA HINCENBERGS, AND KAREN A. HOLBROOK, Depts. of Dermatology, University of California School of Medicine,

San Francisco, CA, and University of Washington School of Medicine, Seattle, WA.

In order to examine skin lipid content during fetal development, frozen human skin samples (gestational ages 50–150 da) (n = 30) were separated into epidermal (E) and dermal (D) fractions with 1 mM EDTA or 10 mM DTT, and the lipid extracts from individual samples were analyzed by quartz rod microchromatography. Before 115 days gestation, E is significantly enriched in alkanes and triglycerides (Tg) while D is significantly enriched in phospholipid and free sterols. Coincident with the development of adipocytes after about 120 days gestation, D, too, becomes enriched with Tg. Simultaneously, E lipid content becomes enriched in sterol/wax esters (SE/WE). Whereas in 110 days epidermis SE/WE content is increased only focally, by 140 days all body sites reflect this change. Thin layer chromatography in (1) hexane; (2) benzene; (3) hexane: ether: acetic acid (70:50:1), showed that the change in this fraction was predominantly due to increased SE content, but the appearance of WE did account for some of the change. These studies demonstrate that distinct differences in E and D lipid content are evident as early in fetal development as adequate E/D separations can be obtained (>70 days). Althouth alkanes have been encountered in normal and pathological E postnatally, their origin has been uncertain. The observation that alkanes are found in significant quantities in fetal E between 50 and 120 days provides further evidence that they are endogenous lipids. The shift to a prominent SE fraction in E correlates morphologically with emergent keratinization of the follicular epidermis.

7. Uptake of Cholesterol Sulfate by Cultured Human Keratinocytes and Fibroblasts. MARY L. WILLIAMS AND MAJA PONEC, Depts. of Dermatology, University of California School of Medicine, San Francisco, CA, and University Hospital, Leiden, The Netherlands.

Cholesterol-cholesterol sulfate (CS) homeostasis may play an important role both in normal desquamation and in the pathogenesis of recessive X-linked ichthyosis. That CS may be an important feedback regulator of epidermal cholesterol synthesis is suggested by: 1) CS inhibition of sterologenesis in cultured cells; and 2) the autonomy of epidermal sterologenesis from serum cholesterol *in vivo*, and in stratifying keratinocyte cultures, which lack low density lipoprotein receptors. To determine whether CS uptake, like cholesterol, is receptor mediated, we prepared from ^{3}H-CS by a modification of the method of Higaki, et al. (J. Vitamin. 11:361, 1965). We corrected for extracellularly entrapped CS and non-specific adsorption with an excluded compound (^{14}C-sucrose) and by rinsing with 3% Triton X-100. Uptake of ^{3}H-CS at several time points between ¼ and 6 hours was linear in both keratinocytes and fibroblasts at 37°C, and was virtually identical at both low (10^{-8} m) and high (10^{-6} to 10^{-5} m) CS concentrations, indicative of a non-saturable, non-receptor-mediated process. Moreover, uptake of CS was similar in both pre-confluent (5 da) and in stratifying (>14 da) keratinocyte cultures, and in both low and high Ca^{++} media. Because of a high degree of non-specific cytosolic binding, the existence of a high-affinity intracellular binding system could not be ascertained. These studies indicate that the highly amphipathic sterol metabolite, CS, readily diffuses into the cell, independent of cell membrane receptors. CS, which is predominantly generated in the upper epidermis, may diffuse to subjacent epidermal layers to regulate sterologenesis.

8. An In Vitro Model for Study of the Role of Lipids in Corneocyte Cohesion and Desquamation. A. W. RANASINGHE, P. W. WERTZ, I. C. MACKENZIE, D. T. DOWNING, AND J. S. STRAUSS, The School of Dentistry and the College of Medicine, University of Iowa, Iowa City, IA.

Mouse ear skin in organ culture remains viable and proliferates at rates comparable to those observed *in vivo* for at least 2 weeks, during which time differentiation, as judged by ultrastructural features, remains normal. In addition, a cohesive stratum corneum is maintained while loosened material accumulates on the surface. Thus, the system closely approaches the *in vivo* pattern of desquamation and offers a model system to study the role of lipids in corneocyte cohesion and desquamation. After 8 days of culture, desquamated material was scraped off, and the cohesive stratum corneum was freed of viable cells by trypsinization and sonication. Lipids were extracted with chloroform:methanol and analyzed by quantitative thin-layer chromatography. No differences were found between the lipids of the cohesive stratum corneum from freshly excised or cultured skin. However, cholesteryl sulfate constituted 4–5% of the polar lipid in cohesive stratum corneum but was reduced to only 0.4% of the polar fraction in the desquamated material. A minor unidentified lipid underwent a similar loss during desquamation, while the ceramide proportions remained unchanged. The present work has a major advantage over previous *in vivo* studies in that the skin surface remains sterile; therefore, possible degradation of cholesteryl sulfate by bacteria is not a factor. Furthermore, the present system permits complete extraction of lipids, which is not possible when intact animals are used. The findings of this study, which is the first to use this organ culture system, support the contention that cholesteryl sulfate serves as an intercellular cement. The model offers potential for use in studies in which factors affecting keratinization are altered.

9. Novel Regulatory Actions of 1α,25-Dihydroxycholecalciferol on the Metabolism of Polyphosphoinositides in Murine Epidermal Keratinocytes. W. TANG, V. A. ZIBOH, R. R. ISSEROFF, AND D. T. MARTINEZ, Dept. of Dermatology, School of Medicine, University of California, Davis, CA

We have previously demonstrated that turnover of cellular polyphosphoinositides (poly-PtdIns) is associated with the onset of differentiation in normal murine keratinocyte cultures. Since 1α,25dihydroxycholecalciferol ($1\alpha,25(OH)_2D_3$) has been reported to enhance morphologic aspects of keratinocyte differentiation, we tested the effects of this agonist on keratinocyte metabolism of poly-PtdIns. Neonatal Balb/C mouse keratinocyte cultures were initiated and plated on DMEM + 10% FCS containing [^{14}C]myo-inositol. After 36 hours, when morphologic evidence of differentiation was first apparent, the radioactive medium was removed and replaced with DMEM + 10% FCS containing 12nM $1\alpha,25(OH)_2D_3$. At specific times after the addition of the $1\alpha,25(OH)_2D_3$, the culture medium was aspirated and analyzed for the distribution of the radiolabel. At six hours, there was a 50% increase in hydrolysis of phosphoinositides in the $1\alpha,25(OH)_2D_3$-treated cells as compared to controls. Further incubation did not result in increased hydrolysis. Analysis of the medium revealed that approximately 60% of the ^{14}C present was in the inositol fraction. Glycerophosphoinositol (GPI) and inositol phosphate (IP) accounted for the remainder of the radioactivity. These results demonstrate that enhanced inositol hydrolysis of polyphosphoinositides accompanies $1\alpha,25(OH)_2D_3$-induced keratinocyte differentiation. This study suggests that $1\alpha,25(OH)_2D_3$ may have the novel effect of inducing inositol hydrolysis, thereby initiating keratinocyte differentiation. Thus, this vitamin metabolite may have additional physiologic effects within the skin.

10. Effect of Histamine on Phosphorylation by Protein Kinases A and C in Normal and Atopic Leukocytes. D. M. TRASK, S. C. CHAN, C. A. HIRSHMAN, AND J. M. HANIFIN, Depts. of Dermatology and Anesthesiology, Oregon Health Sciences University, Portland, OR.

The effects of histamine on protein phosphorylation may be mediated by either cAMP-dependent protein kinase (PKA) or by cAMP-independent, calcium/phospholipid-dependent protein kinase (PKC). We have studied the possibility that histamine-desensitization inhibits activation of PKC by negative regulation via increased PKA phosphorylation in mononuclear leukocytes (MNL) of patients with atopic dermatitis (AD).

Hypaque-Ficoll separated normal or AD MNL, with or without histamine pretreatment (100 pM to 10 μM 37° C for 1 hour), were homogenized in Gey's balanced salt solution or Hank's calcium and magnesium-free buffer (with EGTA and Triton-X) for PKA and PKC, respectively. PKA and PKC activities were assayed by phosphorylation of histone IIA by 32P-ATP in the presence of 5 μM cAMP or of histone IA in the presence of calcium and phosphotidylserine, respectively.

Basal PKA activity in AD MNL was 12.8 ± 3.1 units (n = 8), significantly higher than that of normal MNL (6.4 ± 2.8 units, n = 7). Histamine 1 μM caused 34% increase in normal MNL PKA activity but not in AD MNL. At the same concentration, normal MNL PKC was inhibited and activation occurred only at very low (100 pM) histamine concentrations, yielding a mean 21% increase. PKC in AD MNL was not stimulated by histamine. PKA and PKC activities in MNL from patients with AD are indicative of histamine desensitization. This may also relate to the high basal level of cAMP-dependent phosphorylation in AD cells. The enhanced phosphorylative activity appears to correlate with combined membrane receptor and cyclic nucleotide abnormalities observed in leukocytes of persons with atopic disease.

11. Regulation of Keratin Phosphorylation in Rabbit Corneal Epithelium. A. VIDRICH, K. RYAN, AND I. M. FREEDBERG, Dept. of Dermatology, NYU Medical Center, New York, NY.

In ME180 cells, a human cervical carcinoma line, keratin phosphorylation proceeds via both cyclic AMP-dependent and -independent mechanisms. This dual regulation distinguishes the major acidic keratin from the predominant basic keratin and we have postulated that these keratins subserve different functions within the cytoskeletal network (Gilmartin et al, 1983, J. Cell Biol., 98, 1144). If this hypothesis is true, such dual regulation of keratin phosphorylation should be operative in other epithelia.

We have now examined the phosphorylation of epithelial keratins from cornea, a tissue which can be maintained in organ culture without alteration of its keratin pattern. Agents known to increase intracellular cyclic AMP levels were tested and, after extraction, the keratins were resolved by SDS polyacrylamide gel electrophoresis. Phosphorylation was assessed by autoradiography. As in ME180 cells, the corneal keratin pattern shows one major basic and one major acidic keratin, both being phosphorylated. As predicted, phosphorylation of the corneal acidic keratin was enhanced in the presence of cyclic AMP agonists. Two-dimensional peptide mapping analyses of acidic phosphokeratins from both ME180 cells and corneal epithelium indicate that only a subset of the phosphopeptides is under cyclic AMP regulation.

These data indicate a common mechanism of keratin phosphorylation, operative in normal and pathologic epithelia.

12. Distribution of Caldesmon (a Calmodulin and Actin-Binding Protein) in Cultured Keratinocytes. JUN ADACHI*, YUKIO KITANO*, NATSUKO OKADA*, KENJI SOBUE†, AND SHIRO KAKIUCHI†, *Dept. of Dermatology and †Dept. of Neurochemistry and Neuropharmacology, Institute of High Nervous Activity, Osaka University School of Medicine, Osaka, Japan.

Caldesmon is a calmodulin-binding protein which was discovered in chicken gizzard smooth muscle. This protein is also able to bind to actin filaments. The binding of caldesmon to calmodulin and actin filaments alternates depending upon the concentration of Ca^{2+} (flip-flop binding). Caldesmon is composed of Mr 150K and 147K heterodimer subunits and was demonstrated to be present in bovine aorta, uterus and human platelets. Surprisingly we discovered that a Mr 77K polypeptide was a only protein in cultured cells which cross-reacted with the specific anti-caldesmon antibody. In this study the distribution of caldesmon in the cultured human keratinocytes was investigated by the indirect immunofluorescence technique using anti-caldesmon antibody. Antiserum to chicken gizzard caldesmon was produced in rabbits. The anti-caldesmon IgG fraction was prepared from the serum by ammonium sulfate precipitation followed by affinity chromatography on caldesmon-Sepharose 4B. In the regular medium the keratinocyte showed diffuse distribution of anti-caldesmon immunofluorescence in the cytoplasm. In some cells, the fluorescence was conspicuous along the cellular junction and at the leading edge. When cultured in low Ca^{2+} (0.05 mM) medium, one observed more condensated fluorescence at the leading edge and a faint circular ring along the periphery of the cellular body. SV-40 transformed human keratinocytes had a more diffuse distribution. These findings indicated that caldesmon played a role in the movement and intercellular connection of keratinocytes. The fluorescence pattern seen with anti-actin were similar to that of anti-caldesmon.

13. Induction of Heat Shock Proteins in the Cytoskeletal Fraction of Human Epidermal Cells Following Heart Treatment. ELAINE K. ORENBERG, LINDA M. DICICCO, AND ROBIN L. ANDERSON*, Depts. of Dermatology and *Radiology, Stanford University School of Medicine and Psoriasis Research Institute, Stanford, CA.

Hyperthermia (44°C, 30 min.) clears psoriatic plaques: a single treatment produces reformation of the stratum granulosum indicating normal keratinocyte maturation. After exposure to elevated temperatures (>43°C) many mammalian cells synthesize new proteins called heat shock proteins (hsp's). It is possible that hsp's may be implicated in the normalization of keratinocyte maturation seen in psoriatic plaques.

Epidermal keratinocytes freshly isolated from keratatome slices of adult skin or newborn foreskins (HFK) and cultures of first passage HFK cells were heated (43° ± 0.1°C, 45 min), then pulsed with ^{35}S-methionine (4 hrs., 37°C). Whole cells, triton soluble and insoluble fraction proteins were quantitated, then characterized by one and two-dimensional SDS-PAGE after Coomassie blue staining and new proteins evaluated by autoradiography.

Heat treatment resulted in decreased protein synthesis. Increased amounts of protein in the 67,000 kilodalton (kD) gel band were evident in all heated fresh and cultured cells as compared to controls and associated with the cytoskeletal fraction. Additional bands at 60, 70 and 88 kD appeared in whole cell preparations of heated cultured cells. Two-dimensional gels showed new protein at a site where other mammalian 67 kD hsp's migrate. However, the increased quantities of 67 kD may also represent the increased expression of the 67 kD keratin gene product—a marker of keratinocyte maturation seen in fresh epidermal cells but present in markedly reduced quantities in cultured cells. Since psoriatic plaques show decreased formation of 67-70 kD keratins it may be that heat induction of the 67 kD hsp's, keratin or both are important in the maturation process of epidermal cells in psoriasis.

14. Human Epidermis and Cultured Human Keratinocytes Synthesize a 70 KD Heat Shock Protein in Response to Temperature Elevation to 42°. JOSEPH MCGUIRE AND LUANN LIGHTFOOT, Dept. of Dermatology, Yale University School of Medicine, New Haven, CT.

Temperature elevation induces the selective synthesis of heat shock proteins (HSP) in bacteria, yeast, Drosophila, and mammals. Because the epidermis is the only human organ regularly exposed to 42°, we examined the synthesis of new peptides by cultivated keratinocytes and newborn skin organ cultures maintained at this temperature. Synthesis was measured by the incorporation of ^{35}S-methionine into proteins that were subjected to electrophoresis on 12.5% polyacrylamide gels which were then autoradiographed.

We found that cultivated human keratinocytes synthesize a 70 KD peptide (HSP 70) after exposure to 42° for 1 hour. The synthesis of this peptide decreased 4 hours after the cultures are returned to 37°. The synthesis of the 70 KD peptide is not reduced by either indomethocin or eicosatetraynoic acid, compounds that interfere with arachidonic acid oxidation or by fluocinolone acetonide. Both actinomycin D and cycloheximide prevent the synthesis of HSP-70 in keratinocyte cultures exposed to 42°.

HSP 70 is nearly isomobile with a 69 KD keratin found in differentiated keratinocytes. To establish the relationship between HSP 70 and the 69 KD keratin, the 70 KD HSP was transblotted to nitrocellulose paper and found not to be immunoreactive with antibody to the 69 KD keratin.

Neonatal foreskin placed at 42° synthesizes HSP 70. Although other organs synthesize a variety of HSP's, epidermis and HFEC synthesize predominantly HSP 70.

15. Effects of Retinoids on the Cyclic AMP System of Pig Skin Epidermis. AKIRA OHKAWARA, NORITAKA OHKUMA, AND HAJIME IIZUKA, Dept. of Dermatology, Asahikawa Medical College, Asahikawa, Japan.

Although retinoids reveal various biological and biochemical activities on epidermal keratinocytes, their effect on epidermal cyclic AMP system has been less well characterized. In order to elucidate the relation between them, in vitro pig skin slice system was employed. Pig skin also was obtained by use of a Castroviejo keratome set at 0.2 mm. The slice was cut into 5 × 5 mm squares, and they were floated in RPMI 1640 medium with various retinoids. They were incubated at 37°C in an atmosphere of 5% CO_2 in air. After an appropriate time, skin squares were treated with epinephrine for cyclic AMP accumulations. During the long term (6–24 h) incubation in vitro, control-skin gradually lost its sensitivity to epinephrine. The addition of Ro 10-1670, an active derivative of Ro 10-9359 (etretinate) resulted in rather increased beta-adrenergic adenylate cyclase response of epidermis. The effect was observed at 1 μM concentration and the maximal effect was observed at 10 μM. There was no significant difference in phosphodiesterase activities between control and retinoid-treated skin. The effect was also observed by the addition of all-trans-retinoic acid, retinol, and Ro 10-9359; the latter two compounds revealed much less effects. It has been known that glucocorticoids and colchicine have similar augmentation effects on the beta-adrenergic response of epidermis. The addition of combinations of drugs (Ro 10-1670 & hydrocortisone, Ro 10-1670 & colchicine) resulted in the more marked (additive or synergistic) effects than the single addition of each chemical. Our data indicate that the epidermal beta-adrenergic adenylate cyclase response is modulated by retinoids probably in an independent mechanism stimulated by glucocorticoids or colchicine.

CONCURRENT SESSION 3C: CLINICAL RESEARCH II

Federal

Clark Huff and Masato Niimura, Presiding

1. Additive Effects of Ultraviolet B and Coal Tar on Cutaneous Carcinogen Metabolism. HASAN MUKHTAR, PETER M. MATGOURANIS, BENJAMIN J. DELTITO, JR., MUKUL DAS, AND DAVID R. BICKERS, Depts. of Dermatology, Case Western Reserve University and VA Medical Center, Cleveland, OH.

The Goeckerman regimen is widely used for the treatment of human psoriasis. This modality consists of ultraviolet B (UVB) radiation and crude coal tar (CCT) both of which are known to be carcinogenic for the skin of experimental animals and humans. CCT is rich in polyaromatic hydrocarbons (PAHs) whose cancer-causing effects are thought to be related to their metabolic biotransformation into highly reactive species which bind to DNA. This metabolic biotransformation is carried out by the cytochrome P-450-dependent monooxygenase commonly known as aryl hydrocarbon hydroxylase (AAH). The extent of inducibility of AHH in target tissue appears to correlate with tumor risk. In this study we examined the effect of UVB alone, CCT alone and the combination of UVB and CCT on the inducibility of AHH and other monooxygenases such as 7-ethoxycoumarin O-de-ethylase (ECD) and ethoxyresorufin O-de-ethylase (ERF) activities in the skin of neonatal rodents. Exposure of the animals to UVB (100–1500 mJ/cm^2) alone resulted in a dose-dependent increase (up to 230%) in cutaneous AHH, ECD and ERF activities. A single topical application of CCT (10 ml/kg) 24 hours before sacrifice resulted in significant induction (420–1160%) of AHH, ECD and ERF activities. Treatment of animals with CCT followed by UVB resulted in additive and/or synergistic effects on these enzymes in the skin. However, exposure of animals to UVB prior to CCT application had effects similar to that of CCT alone. The differential responses in AHH activity were found to parallel the capacity of skin enzymes to enhance binding of ^{3}H-benzo(a)pyrene to DNA. These studies indicate that skin exposure to the components of the Goeckerman regimen in neonatal rodents may have an additive effect on enhancing enzymatic activity that is critical to the initiation of a neoplastic response.

2. Differential Alteration of Benzo(a)pyrene Metabolism in Skin and Liver by Hexachlorobenzene. H. F. MERK, K. BOLSEN, C. KUGLER, AND G. GOERZ, Depts. of Dermatology, University of Cologne and University of Duesseldorf, F.R.G.

Initiation of carcinogenesis in skin and other organs by polycyclic hydrocarbons—such as benzo(a)pyrene (BP)—results predominantly from the cytochrome P-450 (P-450)-dependent formation of highly reactive metabolites. Foreign chemicals—such as the porphyrinogenic fungicide hexachlorobenzene (HCB)—may influence significantly the metabolism of polycyclic hydrocarbons by altering the relative proportion and total concentration of various P-450 isoenzymes in microsomes of the skin and other organs. Human adipose tissue contains up to 106 ± 70 ng HCB/g adipose tissue (Williams et al., J Toxicol Environ Hlth 13:19, 1984). Therefore in the present study the effects of orally applied HCB on BP-metabolism in the skin and the liver were investigated.

Female Wistar rats were fed a HCB-containing diet (0.05%). After ten days P-450 content, P-450 dependent 7-ethoxycoumarin O-deethylase activity and aryl hydrocarbon hydroxylase activity in liver and skin and after sixty days the porphyrin, δ-aminolevulinic acid and porphobilinogen contents in the urine were markedly enhanced.

The microsomal dependent BP-metabolism was determined using a high pressure liquid chromatography equipment as recently described (Bickers et al., J Invest Dermatol 83:51, 1984). The metabolism of BP was enhanced markedly in the skin and the liver. However, in the skin the formation of the precarcinogen BP 7,8 diol was enhanced threefold whereas in the liver there was an enhancement not only at the BP 7,8 diol but also a marked enhancement of the phenolic metabolites and a decrease of BP 9,10 diol formation after HCB feeding.

3. Evaluations of Sensitivity and Specificity of IgA Class Antiendomysial Antibodies for Dermatitis Herpetiformis. ERNST H. BEUTNER, TADEUSZ P. CHORZELSKI*, VIJAY KUMAR, AND SUSAN KRASNY, Depts. of Microbiology and Dermatology, SUNY at Buffalo School of Medicine, Buffalo, NY, and *Dept. of Dermatology, Warsaw Medical School, Warsaw, Poland.

Recent reports on the specificity of antiendomysial antibodies (AEmA) for dermatitis, herpetiformis (DH) and celiac disease (Ann. N.Y. Acad. Sci., *420*, 325, 1984; Br. J. Dermatol. *110*, 395, 1984) merit double blind evaluations. Four studies were organized for tests of coded sera using monkey esophagus sections for indirect immunofluorescent tests for the IgA class AEmA reactions with reticular structures of the smooth muscle fibers. The four studies were organized by S. Katz of the NIH in Bethesda, J. Leonard of St. Mary's in London, S. Bean of the Diagnostic Clinic of Houston and M. Meurer of the University of Munich. The sensitivity of the tests for DH patients not on gluten-free diets in these double blind studies was (no. pos./no. DH) 17/33, 8/11, 3/4 and 4/4, i.e. a sensitivity of 61% for all. This does not differ significantly from the sensitivity of 75/104 or 72% reported previously. All DH and celiac disease cases controlled by gluten-free diets (2, 11, 2 & 0 in these four studies) were negative for AEmA. All non-DH cases examined (30, 15, 45 & 4 in these four studies) were also negative for AEmA. This included 5 cases with linear IgA deposits in the skin from the NIH but no active untreated celiac disease cases. (Other studies indicate that celiac disease and DH have similar AEmA frequencies). These findings indicate that tests for IgA class AEmA have a sensitivity for DH of about 66% and a specificity of 100% exclusive of celiac disease.

4. Granular IgA Is Decreased or Absent in Never Involved Skin in Dermatitis Herpetiformis. J. J. ZONE, L. A. CARIOTO, B. A. LASALLE, AND M. J. PETERSEN, Division of Dermatology, University of Utah School of Medicine, Salt Lake City, UT.

Dermatitis herpetiformis (DH) has been said to be characterized by granular IgA in dermal papillae of "uninvolved" skin, implying that IgA is equally distributed in skin independent of previous or current lesions. We have hypothesized that granular IgA deposition is more intense at regional sites where lesions occur.

We obtained punch biopsies from 20 patients with DH. One specimen was taken 1 cm from an active lesion and a 2nd from an area where the subject denied ever having a lesion. IgA was quantified without knowledge of biopsy site, using semi-quantitative immunofluorescent evaluation (0–3+) and computerized photometric analysis of the intensity of fluorescence in the basement membrane zone (BMZ). Computerized fluorescence (CF) correlated well with routine studies (r = .79).

All 20 specimens from perilesional skin revealed granular IgA deposition (x = 1.93 .75) but only 4/20 from never involved skin were positive (x = 0.25 .55). CF was greater in perilesional skin (.522 .389 volts) than never involved skin (−.002 .12 volts) ($p < .001$). IgA was absent in never involved skin of 5/5 untreated patients and in 7/7 additional patients adhering to a strict gluten-free diet.

We conclude that: 1) IgA is not equally distributed in uninvolved skin, 2) clinical misdiagnosis is likely to occur with random biopsy, 3) the relative intensity of fluorescence at the BMZ may be quantified using computerized analysis. Results from earlier studies of "uninvolved skin" in DH may be confounded by actually evaluating residual IgA in previously involved sites.

5. Characterization of the Circulating and Secretory Immune Response to Gliadin in Patients with Dermatitis Herpetiformis (DH). R. P. HALL AND G. WALDBAUER, Dept. of Dermatology, Duke University, Durham, NC.

We have previously demonstrated that IgA in the skin of patients with DH is IgA_1, suggesting it may not be primarily the result of a mucosal immune response. In order to characterize the mucosal immune response we have analyzed sera from 34 patients with DH, 11 patients with linear IgA disease, 10 HLA B8, and 15 non-HLA selected normal subjects for anti-gliadin (gli) antibodies using immunoglobulin (Ig) class specific ELISAs and have determined the IgA subclass composition of the anti-gli response. 12 of 34 (35%) DH patients had IgA anti-gli (2-164 μg IgA/ml; N1 ≤ 1.0 μg IgA/ml). 3 of 33 (9%) DH patients had IgG-anti-gli (5.4–20 μg IgG/ml), all of whom also had IgA-anti-gli. None of the linear IgA patients or HLA B8 normals had IgA or IgG anti-gli. 4 of 28 (14%) DH patients had IgM anti-gli, 2 of whom had IgG or IgA anti-gli. IgA-anti-gli isolated form 8 DH sera using gli-coupled Sepharose 4B was analyzed for IgA subclass composition by ELISA using monoclonal antibodies against IgA_1 and IgA_2. 46–87% (mean 68%) of the IgA-anti-gli was IgA_1 while 13–54% (mean 32%) was IgA_2. This was significantly different from serum IgA subclass composition (mean IgA_1 = 85%, IgA_2 = 15%; $p = .02$). Saliva from 20 DH patients and 7 normal subjects was also tested for anti-gli antibodies. Saliva from DH patients contained a mean concentration of 19.3 μg/ml of IgA and 1.93 mg/ml of total protein (nl:IgA = 19.9 μg/ml; protein = 1.72 mg/ml). IgA, IgG, or IgM anti-gli antibodies, however were not detected in the saliva. Thus mucosal exposure to gli in most DH patients does not result in a salivary or serum antibody response. 35% of DH patients however do develop in the serum a predominantly

IgA anti-gli response with both IgA_1 and IgA_2, which suggests a mucosal origin, and is different from the IgA subclass composition of DH skin.

6. Modification of Crithidia Immunofluorescence Staining and Its Correlation with Farr Assay Technique. TABITHA HENDERSON, THOMAS MEDSGER, RICHARD D. SONTHEIMER, AND J. S. DENG, Dept. of Dermatology and Division of Rheumatology, University of Pittsburgh, Pittsburgh, PA, and Dept. of Dermatology, University of Texas Health Science Center of Dallas, Dallas, TX.

Previously we have demonstrated that certain anti-histone antibodies can bind to Crithidia kinetoplast. This indicated that Crithidia kinetoplast staining is not specific for anti-DNA antibodies. In an attempt to improve the specificity of this test we attempted modification by hydrochloric acid extraction. HCl acid-extracted Crithidia should therefore give positive staining with anti-DNA antibodies only. We screened sera of 212 SLE patients, 15 PSS patients, 25 RA patients, 20 DLE patients, 20 drug-induced LE and 4 Sjogrens patients. 51 of these sera gave positive kinetoplast staining and 31 of these 51 remained positive when tested with the HCl acid extraction method. All of the latter sera were obtained from patients with SLE. Attempts were then made to correlate our Crithidia results with the Farr assay. 36 of the 51 positive Crithidia samples were tested. 22 of the 36 gave positive results by the HCl acid-extracted Crithidia method and 18 of the 22 were positive by the Farr assay. 4 sera were negative by the Farr technique. 14 of these 36 samples were negative on HCl acid-extracted Crithidia smears and none of these were positive on the Farr assay. This indicates a strong correlation between positive Farr assay and positive HCl acid-extracted Crithidia results. However, the finding of 4 positive on HCl acid-extracted Crithidia smears but negative by Farr assay suggests that yet another antibody can bind to HCl acid-extracted Crithidia smears. This again demonstrates the complexity of macromolecules present in the Crithidia kinetoplast and/or the heterogeneity of antibodies in patients with SLE.

7. Subacute Cutaneous Lupus Erythematosus (SCLE) in Multiple Members of a Family with C2 Deficiency. S. J. HODGE*, J. P. CALLEN*, K. B. KULICK†, G. STELZER*, J. J. BUCHINO*, P. COTTER*, C. MACDONALD†, AND D. CROWE*, *University of Louisville School of Medicine, Louisville, KY, and †State University of New York at Buffalo, Buffalo, NY.

Deficiency of the second component of complement (C2d) has been associated with systemic lupus-like syndromes. Immunogenetic studies have shown a close linkage of the C2d and HLA-DR2. Anti-Ro antibodies are present in 75% of the C2d patients with an SLE-like disease. Three members of one family (2 females, 1 male) developed lesions of papulosquamous SCLE and photosensitivity along with mild systemic disease. Six family members were examined for ANA (mouse liver), precipitating antibodies, HLA type, and full complement component analysis.

			HLA					
	Age/Sex	SCLE	A	B	DR	C2	ANA	Anti-Ro
Mother	61/F	−	2, 25	14, 18	1, 2	12	1:20	−
Sib. #1	31/F	+	25, 26	18, —	2, —	None	1:20	−
Sib. #2	30/F	+	25, —	18, —	2, —	None	1:20	+
Sib. #3	29/M	−	25, —	18, —	2,—	None	1:20	−
Sib. #4	26/M	+	25, —	18, —	2, —	None	—	+
Sib. #5	23/M	−	2, —	14, 60	1, 3	13	—	−

All family members were C2 deficient (4 homozygotes, 2 heterozygotes). There appeared to be a strong, but not complete linkage to HLA-DR2, but the serologic reactivity to Ro and ANA did not follow a pattern suggesting linkage to HLA, C2 or clinical disease. All three patients with clinical disease have homozygous C2d. However, one family member with homozygous C2d is clinically healthy, despite a positive ANA. Study of this family has reconfirmed a close linkage of the C2 gene with HLA-A25, B18, DR2, but a lack of linkage of serologic markers with these loci.

8. Electron Microscopic and Immunohistologic Assessment of Isotretinoin Therapy in Cutaneous Lupus Erythematosus. RICHARD C. NEWTON, JOSEPH L. JORIZZO, ALVIN R. SOLOMON, RAMON SANCHEZ, JOHN D. BELL, AND TITO CAVALLO, Dept. of Dermatology, University of Texas Medical Branch, Galveston, TX.

Nine of 10 patients with chronic (CCLE) or subacute (SCLE) cutaneous lupus erythematosus completed 16 weeks of oral isotretinoin therapy (80 mg/day). At baseline the following test results were obtained: B cell counts in peripheral blood (PB) (19.7 ± 1.5; normal 5–15%); OKT4 (T helper subset)/OKT8 (T suppressor/cytotoxic subset) (2.3 ± 0.2; normal ratio 1.5–2.5); routine histology (diagnostic for CCLE or SCLE); immunofluorescence microscopy (IMF) (immunoreactant deposition in the BMZ-9/10 patients); electron microscopy (EM) (varying degrees of basal cell destruction, loss of hemidesmosomes, tonofilament disintegration-10/10 patients); immunoperoxidase assessment of T-cell subsets (OKT3, T4, T8) [most dermal inflammatory cells were OKT3 (Pan T) positive with a 3/2 ratio of OKT4/OKT8].

One patient was lost to followup while 9/9 patients noted an excellent clinical response without side effects which effected therapy. Repeat testing at 16 weeks revealed: no significant change in PB T-cell subsets or B cells; resolving routine histopathology with scarring; negative IMF; normalization of the epidermis on EM: and reduction of all T-cells near the BMZ without change in OKT4/OKT8 ratio. Isotretinoin, a clinically effective short term therapy, emeliorated all assessed pathologic aspects in the 9 CCLE and SCLE patients tested. The primary mechanism of action remains unestablished.

9. T Cell Profiles in Vitiligo. P. E. GRIMES, M. GHONEUM, C. PAYNE, A. P. KELLEY, AND L. J. ALFRED, Division of Dermatology and Dept. of Pathology, King-Drew Medical Center, Los Angeles, CA.

The purpose of this study was to assess T cell profiles in a random group of 10 untreated vitiligo patients and 10 matched healthy controls. The complement mediated cytotoxicity assay was used to determine the percentage of total T, helper and suppressor cells using OKT3, OKT4 and OKT8 monoclonal antibodies (MAb). In brief, peripheral blood lymphocytes (PBL) were prepared by ficoll-hypaque centrifugation. 5 μl of MAb were added to a 50 μl suspension of 1×10^6 PBL and incubated for 10 min. The cells were washed and 100 μl of fresh rabbit complement was added for 45 min at 37°C., followed by addition of 100 μl of trypan blue for 5 min at room temperature. Dead OKT+ cells were enumerated by 0.2% trypan blue dye exclusion. 5 (50%) patients had abnormalities in their T cell profiles. The mean percentages of OKT4 and OKT8 cells were 28 ± 6, 27 ± 6 respectively for this group with an OKT4, OKT8 ratio of 1. However, the remaining patients had only a slight decrease in OKT4 cells of 53 ± 8 and OKT8 of 23 ± 2 with ratios of 2. This data was compared with results from controls with OKT4, OKT8 percentages of 60 ± 3, 33 ± 4 respectively with ratios of 2 ($p < 0.01$). Results were similar using the immunofluorescence assay. Of the 5 patients with abnormal T cell profiles, 20% had associated autoimmune diseases and 40% had autoantibodies present. The helper cell defect observed in these patients tends to support an immunologic pathogenesis of vitiligo.

10. On the Subject of Serum Antibodies to Melanocytes in Vitiligo. GISELA E. MOELLMANN, POLINA KRASS, RUTH HALABAN, ELIZABETH KUKLINSKA, AND AARON B. LERNER, Dept. of Dermatology, Yale University School of Medicine, New Haven, CT.

Hemadsorption techniques were used to test sera from 71 patients with vitiligo and 25 healthy control individuals for the presence of IgG directed against normal human melanocytes *in vitro*. Melanocytes cultured from the foreskins of newborns were used as target cells. In general, patients with extensive vitiligo had significantly higher titers of anti-melanocyte antibodies than did healthy control individuals. Patients who failed to respond to PUVA treatment had higher titers than those who repigmented. In the latter the response to treatment was associated with a drop in antibody titer. The reactivity of the sera was not uniform: sera reacted selectively toward melanocytes from different foreskins, and 50% of the sera that reacted with melanocytes reacted also with fibroblasts derived from the same foreskin. Furthermore, the percentage of cultured fibroblasts that bound autologous antibody doubled when the cells were exposed to TPA, the phorbol-ester that is essential to the growth of normal human melanocytes *in vitro*. A serum with an unusually high anti-melanocyte IgG binding titer was used in pilot immunocytochemical studies to localize sites in skin biopsy specimens. So far two sites, both intracellular, have shown heavy labelling: 1) some melanosomes in melanocytes and keratinocytes; and 2) the contents of the endoplasmic reticulum and nuclear envelope in some basal keratinocytes.

These findings indicate that serum antibodies from patients with active vitiligo also recognize antigenic determinants on cells other than melanocytes; that melanocytes from different donors are not equal in their capacity to bind antibodies; that the expression of cell-surface

antigen may be modulated by growth factors; and that antibody binds to intracellular as well as surface sites.

11. Eccrine Gland Differentiation of a Pluripotent Hair Follicular Tumor in Nude Mice. TAMOTSU KANZAKI, HIKARU ETO, AND SHIGEO NISHIYAMA, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Although a pluripotent tumor is well known to differentiate toward multiple organs, studies in this pluripotence is very scanty. We successfully made an eccrine gland tumor from a hair follicle tumor *in vivo*. We investigated this *in vivo* and *in vitro* transdifferentiation light and electron microscopically and immunohistochemically.

A trichilemmoma cell line, established in 1979 (Cancer Res. 1981), showed a hair follicular differentiation in 1981 in less than 70 passages (Devel. Biol. 1983). These cells started showing an eccrine gland differentiation *in vitro* in 1982 in more than 70 passages (J. Invest. Derm. 1983). When tumors were made in nude mice, the histology was adenoid. Electron microscopically, tumors were those of eccrine gland, i.e., cells made ductal structures with short microvilli and tight junctions. Secretory vesicles were present at luminal sides in cytoplasm. Cells produced basal lamina with half-desmosomes and anchoring fibrilles. Retrospectively, the original tumor and tumors made in nude mice in the early stage were carefully re-examined and revealed to be solid with trichilemmal structures without any evidence of eccrine gland differentiation. Carcinoembryonic antigen was negative in these tumors in the past and present. Monoclonal antibodies to eccrine glands were obtained from this cell line in the stage of hair follicular differentiation used as an immunogen (J. Invest. Derm. 1983). These antibodies strongly reacted with the original tumor as well as tumors in nude mice at present.

These results clearly demonstrated that a pluripotent epidermal appendage tumor was able to differentiate toward a different organ *in vivo* as well as *in vitro*, and suggest that a cell contains the pluripotence from the beginning with or without a phenotypical expression.

12. A Mouse Model for Androchronogenetic Alopecia. J. R. MATIAS, L. MATIAS, V. MALLOY, AND N. ORENTREICH, Dept. of Dermatology, NYU Medical Center and The Animal Sciences Laboratory, Orentreich Foundation, New York, NY.

The development of a convenient animal model is essential to further research on the factors influencing androchronogenetic alopecia (AGA). By selective breeding and routine testing for androgen-mediated cutaneous responses, we have developed a substrain of the B6CBA mouse which expresses AGA when treated exogenously with testosterone (T). Although spontaneous AGA in this model is rare (<1%), alopecia in both sexes was induced either by s.c. T injections (0.1–1.0 mg/day), by feeding a diet of 0.1% methyl-T or by topical application of 5% T-propionate. Alopecia begins as a decrease in hair density along the dorsal neck region after 1 month of daily T injections (1 mg/day). A bald area measuring approximately 1 cm^2 usually forms after 2 months and progresses posteriorly to a maximum of 7 cm^2 after 4 months of therapy. The rate of T-induced alopecia occurred faster in older mice (7–12 months) compared to younger mice (1–3 months). Alopecia also progressed faster in AGA mice treated with dihydrotestosterone. The topical application of 5% cyproterone acetate completely prevented the effect of T. Oral administration of minoxidil, a direct-acting peripheral vasodilator, inhibited the onset of alopecia and caused regrowth of hair in bald mice. These studies indicate that the AGA mouse can be used as a model to examine the mechanisms influencing hair loss, and serve as a tool for the development of novel therapeutic methods.

13. Effects of Promoting and Non-promoting Hyperplasiogens on Epithelial Label-Retaining Cells. I. C. MACKENZIE, J. A. MCCUTCHEON, AND J. R. BICKENBACH, Dows Institute, The University of Iowa, Iowa City, IA.

A subpopulation of epithelial cells which can be marked by its retention of a tritiated thymidine (3HTdR) label may function as epithelial stem cells (Bickenbach, J., J Dent Res 60:1616, 1981) and thus form the target cells for neoplastic transformation. To examine the effects of hyperplasia on such label-retaining cells (LRCs), groups of C3H mice received 1 μCi/gm body wt 3 HTdR at 7:00 am and 7:00 pm on days 10 and 11 following birth. Thirty days later the ears were painted for 3 days with various concentrations (in acetone) of a) the promoting agent 12-0-tetradecanoyl-13-phorbol acetate (TPA) or b) other non- or weakly-promoting hyperplasiogens including mezerein, 4-0 methyl TPA, RPA and hexadecane. One day later treated and control mice received Vinblastine (2 mg/Kg body wt) to arrest cells in metaphase and, 4 hrs later, tissues processed for autoradiography.

Treated tissues were hyperplastic and showed a 2–6 fold increase in cell proliferation. Basal epidermal cells retaining 3HTdR label were present in all specimens. Irrespective of the degree of hyperplasia induced, TPA treated tissues differed from other tissues in that: a) the number of basal LRCs per unit epithelial length was increased, and b) the number of LRCs in metaphase was 4–5 fold higher. If LRCs correspond to stem cells, hyperplasia caused by increased stem cell division would be expected to increase the number of both LRCs and LRCs in mitosis. Thus, by these criteria, TPA appeared to induce hyperplasia by an increased stem cell division, while RPA and other hyperplasiogens induced hyperplasia by increased augmentation division.

14. Cultured Human Epidermal Keratinocytes from Patients with Ichthyosis Vulgaris Are Phenotypically Abnormal. PHILIP FLECKMAN, VIRGINIA P. SYBERT, BEVERLY A. DALE, AND KAREN A. HOLBROOK, Depts. of Medicine (Dermatology), Pediatrics, Periodontics, and Biological Structure, University of Washington and COH Medical Center, Seattle, WA.

Ichthyosis vulgaris (IV) is an autosomal dominant disorder in which keratohyalin granules (KHG) and filaggrin are reduced or gone in the epidermis of affected family members. The phenotype has not been demonstrated in cultured epidermal keratinocytes. Morphological, immunochemical and biochemical markers of epidermal differentiation were studied in keratinocytes from the clinically affected and unaffected skin of four related obligate gene carriers of IV and from a normal adult control. Keratinocytes were obtained by suction blister and cultured by the method of Rheinwald and Green. The cells from affected members formed thickened layers of heaped-up, scaly cells that failed to react with monoclonal antibody (AKH1) to filaggrin and KHG. In contrast, the unaffected gene carrier showed a small granular pattern of fluorescence and normal cells contained large immunoreactive granules. EM confirmed the absence of KHG in affected members and the presence of small or large KHG in unaffected and normal individuals, respectively. Profilaggrin was absent from immunoblots of epidermal extracts of the affected cells and reduced in the cells from the unaffected obligate gene carrier. No differences in keratins were observed among the cultured cells.

We conclude that cultured keratinocytes from patients with IV can maintain major structural and biochemical phenotypic characteristics of the disease. Such an in vitro system offers opportunity for study of the gene defect and for testing the efficacy of therapeutic agents.

15. Detection of Heterozygotes of X-Linked Ichthyosis by Measuring Steroid Sulfatase Activity of Lymphocytes—Mode of Inheritance in Three Families. MASAKI OKANO AND YUKIO KITANO, Dept. of Dermatology, Osaka University, Osaka, Japan.

Recessive X-linked ichthyosis is a metabolic disease in which steroid sulfatase is deficient. The steroid sulfatase locus is located on the short arm of X chromosome and is not subject to X chromosome inactivation. Steroid sulfatase activity is lower in male than in female, and in heterozygote the activity is expected to be lower than normal female. This study was done to investigate the usefulness of determination of steroid sulfatase activity of peripheral blood lymphocytes for the detection of a carrier and to delineate the inheritance in the family. Steroid sulfatase activity was assayed in lymphocytes from 10 ml of heparinized blood obtained from all available members of three families in which X-linked ichthyosis had frequently developed using dehydroepiandrosteronesulfate as a substrate with a modified method of Epstein et al. Lymphocytes of all the patients lacked steroid sulfatase activity and those from the carriers showed significantly lower activity of the enzyme (72.9–103.1 pmol/mg protein/h), while those of all the other normal members examined had normal male (123.9 ± 18.8 pmol/mg protein/h, n = 13) or female activity (187.8 ± 21.0 pmol/mg protein/h, n = 17). In addition, three unmarried females in the families were shown to be carriers of the disease in view of their lower female enzymic level. We conclude that the detection of a carrier of X-linked ichthyosis is possible by measuring steroid sulfatase activity of lymphocytes which are obtained most easily.

CONCURRENT SESSION 3D: PHOTOBIOLOGY

South American

Henry Lim and Itsuro Matsuo, Presiding

1. Oxygen Intermediates Are Involved in Contact Photosensitization, but Not in Ordinary Contact Sensitization to TCSA.

YOSHIKI MIYACHI, SADAO IMAMURA, YUKIE NIWA, YOSHIKI TOKURA, AND MASAHIRO TAKIGAWA, Dept. of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Niwa Institute for Immunology, Kochi, and Dept. of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan.

We have postulated that at least two different subsets of antigen-reactive T cells, one mediating contact photosensitivity (CPS) and the other mediating ordinary contact sensitivity (CS) develop in photosensitized mice. This suggests that both photoactivated TCSA- and ordinary TCSA-skin conjugates are formed after epicutaneous painting of TCSA and black light irradiation indicating the discriminative sensitization process between CPS and CS. In the present study, we investigated the participation of oxygen intermediates (OIs) in the photosensitization process to TCSA by scavenging OIs using long acting liposomal superoxide dismutase (L-SOD) asking if the photodynamic action, i.e., the oxygen-dependent action of light, plays an important role in cutaneous photosensitization using our mouse model. Mice were treated with L-SOD before photosensitization and/or photoelicitation. CPS to TCSA was significantly suppressed by L-SOD treatment. Free SOD or empty liposomes did not have this effect. Adoptive transfer study and cyclophosphamide pretreatment experiment indicated that this suppression was not mediated by suppressor cells. L-SOD treatment induced the suppression of CPS to TCSA, but not CS to TCSA or DNFB, suggesting that the production of photoallergen is more critically dependent on the presence of OIs than that of ordinary contact allergen. In addition to a free radical mechanism, we would like to propose another possible mechanism in which OIs are produced by light absorption in the presence or oxygen and react with the biological substrate to form stable photoproducts inducing photosensitization.

2. Passive Transfer of Photosensitivity by Intradermal Injection of Human Vesiculobullous Lupus Erythematosus Serum and Ultraviolet Irradiation in Guinea Pig. DAVIDE IACOBELLI, LUCA BIANCHI, TORU FUKAYA, AND KEN HASHIMOTO, Dept. of Dermatology, Wayne State University, Detroit, MI, and VA Medical Center, Allen Park, MI.

We studied the effect of intradermal injections of vesiculobullous lupus erythematosus (VLE) serum followed by ultraviolet B (UVB) irradiations in strain 2 guinea pig (GP). Two patients presented with vesiculobullous lesions mostly on sun-exposed areas. The diagnosis of the VLE was made according to the ARA criteria and confirmed by dermatitis herpetiformis-like histology, direct immunofluorescence (IF) consistent with dermo-epidermal junction deposits of IgG, IgA, IgM and C_3, high ANA titers and immunoelectronmicroscopy showing immune complex deposits below the lamina densa. 0.1 cc of each patient's serum was injected intradermally in four different areas of the GP back. Injections were repeated three times, once every 48 h. Two of the injected areas were irradiated with 80 mJ/cm^2 (1 minimal erythema dose) of UVB, λ310 soon after. 96 h after the combined (injection and UVB) procedures erythema and crusting were seen in the areas so treated, whereas the areas that received only serum injections showed slight redness and desquamation. The areas irradiated alone showed 1+ erythema at 24 h. Biopsies were obtained at 24, 48, 72, 96 h. Direct IF with anti-human IgG, IgA, IgM and C_3 was performed on each biopsy. Chronological progression of positivity from the dermis up to the higher epidermal layers in a trans-epidermal elimination-like fashion was seen in the areas injected and irradiated. Upper epidermal deposition corresponded with erythema and crusting. Direct IF of the areas injected only, showed diffuse, non specific dermal positivity. These findings suggest that VLE photosensitivity is due to the combined action of UVL and transferable serum factor(s) and can be reproduced in GP by VLE serum injections and subsequent UVL irradiations.

3. Induction of Porphyrin Photosensitivity in Mast Cell Deficient Mice. CRAIG A. ELMETS, Dept. of Dermatology, Case Western Reserve University, Cleveland, OH.

Cutaneous photosensitivity is a prominent clinical manifestation in patients with porphyria and in patients treated with hematoporphyrin derivative (HpD) photochemotherapy. The mechanism of this abnormal sensitivity to light has not been completely elucidated. The purpose of this study was to examine in a murine model the role of vasoactive amines and tissue mast cells in the development of cutaneous photosensitivity caused by porphyrins. Porphyrin photosensitization was produced by inoculating mice with 10 mg/kg HpD and irradiating them 6 hours later with 1 J/cm^2 405 nm. radiation from a filtered high pressure mercury arc lamp. The amount of ear swelling present immediately after irradiation served as an index of the degree of photosensitization which had occurred. The administration of reserpine, a serotonin depleting agent, to C3H mice prior to HpD inoculation and light exposure suppressed the ear swelling response 50 to 90%. Similar results were obtained in mice pre-treated with either of 2 serotonin receptor antagonists, cyproheptadine or methysergide. Mast cell degranulation was a prominent feature observed in histologic sections of ears taken from mice treated with HpD and light. However, the ear swelling response could readily be elicited in W/W^v mast cell deficient mice. When these mast cell deficient mice were pre-treated with cyproheptadine, ear swelling was inhibited by 85%. These studies indicate that vasoactive amines play a fundamental role in the production of porphyrin photosensitivity. The elicitation of porphyrin photosensitization in mast cell deficient mice suggests that other cell types besides mast cells are important in the production of this phototoxic lesion, and that these other cells produce their effect independent of mast cells.

4. Photosensitized Destruction of Normal and Leukemic T Cells Using Monoclonal Antibody (MAb)-Directed Hematoporphyrin (HP). A. R. OSEROFF, J. WIMBERLY, C. LEE, V. ALVAREZ, AND J. A. PARRISH, Dept. of Dermatology, Massachusetts General Hospital, Boston, MA, and Cytogen Corporation, Princeton, NJ.

Antibody-mediated photolysis (AMPL), using monoclonal antibodies (MAbs) to localize photosensitizers at specific target cells, may improve the efficacy of MAb serotherapy. To evaluate this new approach, HP-dihydrazide was conjugated to the oxidized carbohydrate moiety of anti-T cell MAbs (anti-Leu-1, 2a, 3a) or non-specific control MAb. Conjugation ratios of 0.85–3 HP/MAb were achieved, with no change in MAb binding specificity or in HP photosensitized $^1O_2$ production as measured by the RNO bleaching technique. Conjugates were tested on the human HPB-ALL T cell line and on PHA stimulated lymphocytes using 360–410 nm irradiation. Light-dependent cell killing was observed for all three MAbs; non-specific MAb-HP or anti-T cell MAb alone had no effect. For anti-Leu-1-HP (2 HP/MAb), there was >90% decrease in thymidine incorporation compared to controls at 50 J/cm^2. About 10^{-15} gm HP were bound and about 10^8 photons absorbed per cell killed. >80% of MAb-HP binding was preserved after irradiation. There was an inverse relationship between conjugation ratio and cytotoxic light dose. Irradiations in D_2O gave a 3-fold enhancement of phototoxicity, consistent with a $^1O_2$ process. AMPL has potential advantages over other immunotherapies in that killing depends on both MAb binding and light so the MAb need not be completely tumor specific. The conjugates need not be internalized, may be effective after being shed, and may also kill neighboring antigen-negative tumor cells. AMPL has promise for cutaneous T cell lymphoma and other diseases accessible to light.

5. Human Protoporphyria: Effects of Liver Failure on Porphyrin Distribution Dynamics. M. B. POH-FITZPATRICK, R. T. WHITLOCK, AND J. H. LEFKOWITCH, Depts. of Dermatology, Medicine, and Pathology, Columbia University, New York, NY.

A rare opportunity to experimentally observe effects of liver failure on protoporphyrin (PP) distribution in human protoporphyria was provided by an episode of severe cholestatic viral hepatitis in a patient with well established stable basal [PP] in rbc, plasma and feces. Profound alterations in PP partitioning ($\bar{x} \pm$ SD, n) and excretion efficiency ($\bar{x}$ 24 h fecal PP excretion/$\bar{x}$ PP retained in total rbc mass) during peak hepatic dysfunction are shown in the Table:

	μg PP / dl rbc	μg PP / dl plasma	μg PP / 24h feces	$\bar{x}$ μg PP / rbc mass	$\bar{x}$ 24h fecal PP / $\bar{x}$ rbc mass PP
Baseline: (3 years)	1447±463,6	62±14,6	4524±3326,6	21996	0.208
Episode: (14 days)	6436±758,7	244±251,7	3249*	67038	0.049
Recovery: (4 months)	1503±191,9	49±13,9	2848±2023,8	22229	0.128

* Total PP content in all feces collected over 12 days ÷ 12.

The slope of the cumulative distribution function for increasing cell age vs. rbc [PP] changed from sharply negative to flat during failure, indicating retardation of transmembrane efflux of rbc PP against rising plasma [PP]. As liver function improved and plasma [PP] fell, rbc PP efflux increased and the slope reverted to sharply negative, indicating the liver was clearing PP effectively.

These observations 1) confirm hypothetical predictions of such effects (*Gastroenterology* 79:115, 1980) and 2) support the associated theory that serial monitoring of PP distribution in individual patients may allow earlier detection of impending hepatic decompensation.

6. Differential Effect of Protoporphyrin and Uroporphyrin on Mast Cells: Correlation with Clinical Manifestations of Erythropoietic Protoporphyria and Porphyria Cutanea Tarda. HENRY W. LIM, STEPHEN I. WASSERMAN, AND IRMA GIGLI, Divisions of Dermatology and Rheumatology, University of California, San Diego, CA.

Upon exposure to sunlight, patients with erythropoietic protoporphyria (EPP) develop burning, stinging, erythema and edema, while patients with porphyria cutanea tarda (PCT) present with skin fragility and vesicles. Since the predominant porphyrins are protoporphyrin (PP) and uroporphyrin (URO) respectively, we investigated if the different clinical manifestations could be in part due to the different effect of PP and URO on cells in the skin, particularly the mast cell. Rat serosal mast cells were purified to 95% purity and labelled with ^{3}H-serotonin; its release, which closely correlates that of histamine, was used as a measurement of mast cell degranulation. In the presence of PP (25-500 ng/ml), exposure of mast cells to 400–410 nm irradiation (0.00045 to 0.16 J/cm^2) resulted in a Ca^{++}-independent release of ^{3}H-serotonin (up to 44.5%). This release was dependent on the PP concentration and irradiation dose. Degranulation was further increased by subsequent exposure of these cells to Ca^{++}-ionophores, suggesting a distinct mechanism of activation induced by PP and light. Following irradiation of mast cells (98% purity) labelled with ^{14}C-arachidonic acid (AA), a PP-dose dependent net release of ^{14}C-AA was also noted (up to 15.9%). In contrast, irradiation of mast cells in the presence of URO (up to 4000 ng/ml) resulted in <5% net release of ^{3}H-serotonin, and <3% release of ^{14}C-AA. In all experiments cell viability was >95% and irradiation, PP, or URO alone was without effect. This differential effect of PP and URO on mast cells may partly account for the distinct cutaneous manifestations seen in patients with EPP and PCT.

7. Nuclear DNA Endonuclease Activities on Ultraviolet Light Irradiated DNA in Normal and Xeroderma Pigmentosum Lymphoblastoid Cells. MURIEL W. LAMBERT, Dept. of Pathology, UMDNJ-New Jersey Medical School, Newark, NJ.

Nuclear DNA endonuclease activities from normal human (GM 1984, GM 3299) (NL) and xeroderma pigmentosum, complementation group A (GM 2345, GM 2250A) (XPA) lymphoblastoid cells on 254 nM ultraviolet light (UVC) irradiated DNA were compared. Chromatin-associated DNA endonucleases were isolated from all cell lines and subjected to isoelectric focusing. Each isoelectric focusing fraction was assayed for DNA endonuclease activity using calf thymus DNA as substrate. Peaks of activity were pooled and assayed for activity on circular, supercoiled PM2 bacteriophage DNA which was irradiated at 254 nm with a mineralight UV lamp (Ultra-Violet Products, Inc.) at a total UV dose of 90 to 1080 J/m^2. Enzyme-treated and control PM2 DNA were analyzed by electrophoresis on 0.9% agarose gels. In both normal and XPA cell lines there is an endonuclease fraction at pl 7.6 ± 0.2 which is more active on UVC irradiated (180 J/m^2) DNA than on native DNA. The levels of this endonuclease activity between XPA and normal cells was similar. As the UVC dose was increased to 1080 J/m^2 those endonucleases, pls 9.2 and 9.8, which are active on apurinic/apyrimidinic DNA showed activity on the irradiated DNA. These results indicate that there is a UV endonuclease activity at pl 7.6 in normal human cells and that this activity is not defective in XPA cells.

8. Wavelength Dependence for Gilvocarcin V Effects on Superhelical pBR322 DNA. R. M. KNOBLER, F. P. GASPARRO, W. A. SAFFRAN, AND R. L. EDELSON, Dept. of Dermatology, Columbia University, New York, NY.

The activity of Gilvocarcin V (GV), an antitumor antibiotic is enhanced by photoactivation with UVA and visible light. Its potential use as a photochemotherapeutic agent led us to study its effects on DNA. To determine the affinity for DNA, GV fluorescence spectra were recorded in the presence of increasing amounts of DNA. A GV-DNA association constant of 4.6×10^6 was computed using a Scatchard analysis. Since DNA interacts with GV 700 times more strongly than with 8-methoxypsoralen (8-MOP), far less GV is required for the same level of interaction. Furthermore, a shift in the superhelical density of GV treated plasmid DNA analyzed in a chloroquine containing agarose gel revealed that GV intercalates with DNA, indicating that GV can interact with DNA in a specific orientation, facilitating its photochemical modification of DNA. To study the effects of GV, solutions of superhelical pBR322 DNA (25 μg/ml) and GV (250 ng/ml) were irradiated with subbands of light centered at 370, 382, 394, 406, 418 and 430 nm, with total doses up to 3 J/cm^2. The extent of GV plus light-induced DNA strand breakage (nicking) in superhelical DNA was determined by agarose gel electrophoresis. On agarose gels, nicked and undamaged DNA migrate as discrete bands. At each wavelength, the densitometric scans for four time points showed a light-dose dependent increase in the extent of nicked DNA. The wavelength dependence correlated with the absorption spectrum of GV. Optimal nicking occurred at 406 nm, the absorption maximum for GV, where 22% of the DNA molecules had been nicked. GV may be used with less toxic, longer wavelength photons and appears to be a new candidate for photochemotherapy.

9. One Thymine Dimer Inactivates Expression of a Transfected Gene in Xeroderma Pigmentosum Cells. M. PROCTIĆ-SABLJIĆ AND K. H. KRAEMER, Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD.

We have developed a new host-cell reactivation assay of DNA repair utilizing UV-irradiated plasmid vectors. The assay primarily reflects transcriptional activity of DNA transfected into cultured cells. Transcriptional activity is measured as enzyme activity of the transfected genes. UV-irradiated plasmids pSV2catSVgpt (7.3 kb), pSV2cat (5 kb), and pRSVcat (5.2 kb) containing the gene (cat) coding for chloramphenicol acetyltransferase (CAT) were used. 48 hr after transfection cells were lysed and CAT activity was measured by TLC. UV-induced dimer formation in the plasmids was quantitated by the disappearance of form I DNA on agarose gels following irradiation and T4-endonuclease V treatment. In comparison to normal cells, xeroderma pigmentosum (XP) cells (3 complementation group A lines and 1 group D line) expressed the UV-damaged cat gene at much lower levels than the non-irradiated cat gene. Ataxia telangiectasia, retinoblastoma and Lesch-Nyhan lines had expression similar to the normal cells. The D_{37} of the CAT inactivation curve was 58 J/m^2 for pSV2catSVgpt, 48 J/m^2 for pSV2cat and 51 J/m^2 for pRSVcat in XP12BE(SV40) cells and about 860 J/m^2 in normal cells (GM0637(SV40)). The similarity of the D_{37} data in these XP group A cells for 3 plasmids of different size and promoters suggests they all have similar UV-inactivation target size. This data indicates that in the XP group A cells, one UV-induced dimer inactivates a target of about 1.8–2.0 kb, the suggested size of the cat transcriptional unit. This study demonstrates that XP cells are markedly defective in transcription of UV-damaged transfecting DNA. The transcriptional activity of damaged recombinant DNA expression vectors may be useful for assessing repair of DNA in human cells.

10. Growth Related Variations in the Glycosaminoglycan Synthesis of Ultraviolet Radiation Induced Cutaneous Fibrosarcoma Cells. A. LINKER, M. PIEPKORN, AND H. CARNEY, Depts. of Medicine and Pathology, and Veterans Administration Hospital, University of Utah, Salt Lake City, UT.

Murine cutaneous fibrosarcomas induced with ultraviolet radiation provide a model in which to study tumor cell populations which differ in tumorigenic potential. Previously, we found that cells from a cloned regressor tumor labelled in vitro for glycosaminoglycan (GAG) content with ^{35}S and ^{3}H-glucosamine display a 4-fold increase in cell surface-associated sulfated GAGs and a reversal in the heparan sulfate: chondroitin sulfate ratio as cell populations switch from exponential growth to stationary growth at increasing cell densities. We now report that shifts in the hyaluronic acid and sulfated GAG content of various cellular and extracellular matrix compartments occur under the two growth conditions. Hyaluronic acid comprises 21% of total cellular GAGs during exponential growth (>85% of cells in S + G2 + M cell cycle phases), but only 6% in growth-arrested cultures (>50% of cells in G1), due largely to its reduction within the substratum attached material (SAM, material on the flask surface after EGTA-mediated cell detachment) and the pericellular matrix (material loosely associated with cells which is released into the supernatant upon cell detachment with EGTA). Furthermore, heparan sulfate and the chondroitin sulfates concurrently shift from the SAM and the cytoplasmic pool to the cell surface-associated compartment and the pericellular matrix. Thus, differences in GAG quantities, compositional ratios and compartmental distributions occur in fibrosarcoma cell populations differing in rates of growth consequent to cell density. These correlations

suggest that GAGs are involved in some aspect of the control of cell proliferation.

11. Condition and Mechanisms for Giant Cell Formation by 8-Methoxypsoralen Phototoxic Reaction. KAORU WATANABE, TOKIO NUMATA, AND NOBUYUKI MIZUNO, Dept. of Dermatology, Nagoya City University Medical School, Nagoya, Japan.

A probable risk of carcinogenesis in the 8-methoxypsoralen (8MOP)-PUVA treatment has been discussed, but the actual rate of skin cancer following this treatment is very low. The reason remains unknown. Cultured cells treated with 8MOP-PUVA induced not only cell death but also giant cells (GCs) as mutants. It is an important question whether the mutants have a potential for carcinogenesis. In order to assess the above possibility, conditions for GCs formation and their fate were studied. L-929 cells were incubated in the medium containing 10 μg/ml of 8 MOP for 30 min. followed by UVA irradiation, and then cultured for two to eight days. 1) The GCs formation rate reached its maximum when the UVA dose was $1.0 \times 10^3 J/m^2$. Under this condition the colony formation rate of non-GCs was about 1%, while GCs themselves could not form colonies. 2) Two types of GCs were observed, a mononuclear GC and a polynuclear GC. By irradiation with high UVA dose ($2 \times 10^3 J/m^2$), most of the GCs were found to be mononuclear GC throughout the observed period. 3) On the other hand, low dose irradiation ($1 \times 10^3 J/m^2$) produced mostly mononuclear GCs for the first three days, then polynuclear GCs increased. 4) By cinematographic observation it was confirmed that both mononuclear GCs and polynuclear GCs never make cell division, and GCs are formed by inhibition of the cell division process. After entering into pseudo-M-phase, some of these GCs were able to undergo nuclear division and formed polynuclear GCs. Others remained in mononuclear GCs even after pseudo-M-phase. Both mononuclear GCs and polynuclear GCs could not divide, so they do not proliferate and cannot be related to the carcinogenesis by 8MOP-PUVA.

12. β-Carotene Protection Against Psoralen Phototoxicity. ALBERT GILES, ANDRIJA KORNHAUSER, AND WAYNE WAMER, Division of Toxicology, Food and Drug Administration, Washington, DC.

β-Carotene (car) is a naturally occurring antioxidant currently being tested by several investigators for its ability to protect against carcinogenesis. In this study, we attempted to determine whether car protects against psoralen phototoxicity (PT). Groups of albino female Osborne Mendel rats were fed standard, placebo or car fortified diets. After 13 weeks on their respective diets, the backs of selected animals were clipped. The animals were orally dosed with 20 mg/kg 8-methoxypsoralen (8-MOP) in corn oil and irradiated two hours later with 5 J/cm^2 UVA. A significant car protective effect was observed against 8-MOP PT as measured by erythema production. The greatest protective effect was observed in those animals with the highest car skin levels, determined by HPLC. To our knowledge, this is the first demonstration of dietary car protection against psoralen PT. No protection was observed against UVB (0.7 J/cm^2) induced erythema. The mechanism for the observed protective effect against 8-MOP PT remains to be elaborated. Recently, it has been suggested that 8-MOP PT involves a photooxidative mechanism. Car may therefore protect by quenching activated oxygen intermediates; a mechanism similar to that proposed for car protection against photosensitivity in patients with erythropoietic protoporphyria. Alternatively, car may protect against 8-MOP photoaddition to DNA. Although the relationship between car protection against 8-MOP PT and photocarcinogenesis is unclear at present, there is evidence that car protects against both UVB and chemically (DMBA) induced carcinogenesis (Mathews-Roth, Oncology 39:33, 1982). The animal model developed here will facilitate future studies on the protective effect of dietary car against both PT and photocarcinogenesis.

13. Studies on the Mechanism of the Development of Photosensitive Syndrome by Pheophorbide A. SHUICHI KIMURA, TOSHIHIKO ISOBE, HIROAKI SAI, YUJI TAKAHASHI, AND HUMIO INABA*, Laboratory of Nutrition, Faculty of Agriculture, Tohoku University, and *Section of Quantum Electronics, Research Institute of Electric Communication, Tohoku University, Sendai, Japan.

Pheophorbide a, which is one of the decomposition products from chlorophyll, is known to cause photosensitivity in both domestic animals and human. Several years ago in Japan, photosensitive dermatitis has been found to occur in people who had injested large quantities of chlorella tablets produced by certain company. This syndrome is ascribed to the large amounts of pheophorbide contained in some lots of these tablets. However, little was known about the mechanism of the development of this photosensitivity. Therefore, we have studied this from biochemical and physiological view points. We have developed some bioassay systems using blood cells and cultured myocardial cells obtained from the heart of chick embryos. We have shown that in the presence of pheophorbide a, visible light irradiation leads to the production of an active oxygen. This, in turn, results in cell damage by oxidation of lipids, especially in the cell membranes. We attempted to determine the species of active oxygen. Near IR emmision spectra of singlet oxygen produced by pheophorbide a-photosensitized reaction (at 1.27 μm $'\Delta g(0) \rightarrow 3\Sigma\bar{g}(0)$) was determined. Experimental results indicated that the species of active oxygen participated primarily in this reaction was singlet oxygen.

14. Monoclonal Antibodies That Recognize 8-Methoxypsoralen Photoadducts in DNA. R. M. SANTELLA, N. DHAMARAJA, F. P. GASPARRO, AND R. L. EDELSON, Columbia University, New York, NY.

To quantitate in vivo modification of DNA by 8-methoxypsoralen (8-MOP), a panel of monoclonal antibodies that react specifically with DNA irradiated with UVA light (320–400 nm) in the presence of 8-MOP is being developed. Balb c/Cr mice were immunized with DNA modified to an extent of 5.5% with 8-MOP and complexed electrostatically with methylated BSA. A total of 14 stable clones specific for 8-MOP photoadducts were produced, four of which were extensively characterized. The specificity and sensitivity of the antibodies were characterized by enzyme linked immunoabsorbent assays (ELISA). In noncompetitive assays with increasing amounts of 8-MOP-DNA, as little as 0.2 ng (3.4 femtomole 8-MOP) of modified DNA could be differentiated from nonmodified DNA. In competitive ELISA the 50% inhibition of antibody binding to 8-MOP-DNA coated plates ranged from 17 to 67 femtomole 8-MOP photoadducts. Competitive assays with 8-MOP modified poly(dA.dT) (containing only monoadducts) and 8-MOP-modified poly(dAdT.dTdA) indicate that all four antibodies have a high specificity for monoadducts. One of the antibodies was used to assay HPLC fractions from enzymatically hydrolyzed 8-MOP-poly(dAdT.dAdT). The greatest inhibition occurred in those fractions containing the 4′,5′-monoadduct suggesting that this is its primary specificity. DNA was also isolated from myeloma cells that had been irradiated in the presence of 8-MOP (500 ng/ml). Quantitation of adduct levels by ELISA revealed that 3.2 8-MOP molecules were bound per 10^6 bases. Thus the sensitivity of these antibodies should allow their use for the quantitation of adduct formation in human cells exposed to psoralen plus UVA light.

15. The Nature of Melanin Pigmentation Stimulated by Khellin and Psoralens. M. A. PATHAK, Z. ZAREBSKA, J. H. ADDY, AND T. B. FITZPATRICK, Dept. of Dermatology, Harvard Medical School, Boston, MA.

Recent reports indicate khellin (KHE), a furanochromone, to stimulate repigmentation with success in vitiligo patients. We investigated the primary photochemical effect of KHE on melanocytes (MC) and keratinocytes (KC) of mammalian skin and compared its activity to certain furocoumarins [8-MOP, 5-methoxypsoralen (5-MOP) and methylangelicins (MEA)]. *In vitro* and *in vivo* topical effects of 8-MOP, 5-MOP, MEA and KHE, with and without UVA irradiation (2–12 J/cm^2), were studied in punch biopsies and isolated epidermal sheets from pigmented guinea pigs and post-operational human skin incubated in 0.1% 3,4-dihydroxyphenylalanine. A sensitive, indirect immunofluorescent (IF) method with specific immune serum anti-DNA-psoralen photoadduct has been used to detect photochemical conjugation and damage in nuclei of MC and KC. Our results indicate: 1) KHE is a nonerythemogenic agent that photoconjugates with nuclear DNA of MC and KC in a manner similar to photoconjugation of 8-MOP, 5-MOP, or MEA, but to a lesser degree than 8-MOP or MEA. 2) IF staining with furocoumarins was greater in KC and MC than KHE, and more in proliferative KC and MC than in quiescent cells. 3) Threshold dose for detection of IF stain in MC was approximately ten fold for adjacent KC (e.g., 400 ng 8-MOP + 6 J/cm^2 UVA *in vitro* tests and 100 μg 8-MOP + 22 J/cm^2 UVA *in vivo* tests). 4) On a molar basis, the order of activity for stimulation of melanin pigmentation was: trimethylpsoralen > 8-MOP > 5-MOP > 5-MEA ≥ KHE. Stimulation of melanin pigmentation by these agents appears to result from photodamaged melanocytes undergoing DNA repair, mitosis, and subsequent proliferation involving concomitant increased production, melanization, and transfer of melanosomes to KC.

7:30 PM Sheraton Washington Exhibit Hall
COMBINED POSTER SESSION
JOINT SID/JSID-AFCR/ASCI/AAP SESSION

1. The Role of the Aminopropeptides of Type I and Type III Procollagen in 'In Vitro' Fibrillogenesis. R. G. PHELPS, J. S. PERLISH, AND R. FLEISCHMAJER, Dept. of Dermatology, Mt. Sinai School of Medicine, New York, NY.

Previous immunoelectron microscopic studies of human and chick embryo skin have shown that the aminopropeptides of types I and III procollagen are incorporated into small collagen fibrils (<40 nm.) To determine whether this mechanism occurs in vitro, early passage human dermal fibroblasts were grown in ascorbate, and aminopropeptides were studied by immunofluorescence, immunoelectron microscopic, and immunoblotting techniques. Immunofluorescence examinations were performed on early stage (<7 days) and later stage cultures (11, 13 days) using permeabilizing and nonpermeabilizing techniques. The aminopropeptide of type I procollagen showed constant abundant intracellular localization and progressive deposition in the matrix with time. This appeared as a fine fibrillary array overlying the cells and a coarser fibrillar pattern between the cells. The aminopropeptide of type III showed a similar pattern, but with less extensive extracellular distribution. Immunoblotting studies of homogenates of the cell layer, confirmed the presence of collagens containing the aminopropeptide of type I (pN_I) and the aminopropeptide of type III (pN_{III}). Preliminary immunoelectron microscopic studies using the indirect immunoferritin technique suggested that small 10–20 nm fibrils showed labelling for both the aminopropeptides of types I and III procollagen. These data suggest that the aminopropeptides of types I and III procollagen form a part of thin fibrils and may play a role in fibrillogenesis in vitro.

2. DNA-Cytophotometry on Touch Imprints of Lymph Node Biopsies from Patients with Cutaneous T-Cell Lymphoma. STUART R. LESSIN, ERIC C. VONDERHEID, GARY L. GROVE, AND LAWRENCE DIAMOND, Dept. of Dermatology, University of Pennsylvania and Skin and Cancer Hospital, Philadelphia, PA.

The objective of this study was to determine the role of DNA-cytophotometry as a means of augmenting the histopathologic evaluation of lymph node (LN) specimens obtained from patients with cutaneous T-cell lymphoma (CTCL). Touch imprints of 26 LNs from 25 patients with CTCL were fixed in methanol-glacial acetic acid and stained by the Feulgen technique. Scanning DNA-cytophotometry was performed on 100 randomly selected nuclei from each specimen without knowledge of the histopathological diagnosis.

14 LNs showed no histopathological involvement with CTCL and DNA-histograms revealed no evidence of polyploidy i.e. hypertetraploidy. 12 LNs from 11 patients had histopathologic involvement with CTCL with variable effacement of nodal architecture. An abnormal DNA histogram with evidence of polyploidy was demonstrated in 9 of the 12 LNs. One LN had a DNA distribution characteristic of a relatively high level of cell proliferation, but without definite polyploidy. Cytogenetic studies on the blood of this patient demonstrated a clone of lymphocytes with a pseudodiploid karyotype. The remaining two LNs had normal DNA histograms; these LNs were only focally involved with CTCL.

These observations indicate that DNA-cytophotometry correlates well to histopathologic findings in LNs diffusely involved with CTCL, but may be normal in LNs with focal involvement or those that contain cytogenetically abnormal cells that lack evidence of polyploidy.

3. Relationship Between Circulating Anti Ro/SSA Antibody (a-Ro) Levels and Skin Disease Activity in Subacute Cutaneous Lupus Erythematosus (SCLE). S. M. PURCELL, T. S. LIEU, B. M. DAVIS, AND R. D. SONTHEIMER, Depts. of Dermatology, UTHSCD, Dallas, TX, and Wilford Hall USAF, San Antonio, TX.

If a-Ro plays a pathogenetic role in the development of SCLE skin lesions as has been suggested by some workers, one might expect a positive correlation between a-Ro levels and skin disease activity in SCLE. To investigate this possibility we have examined the relationship between a-Ro levels and skin disease activity in 12 SCLE patients (pts) whom we had examined and collected blood from on at least 3 separate occasions. A-Ro levels were determined in 80 serum specimens by double immunodiffusion (ID) using Wil-2 cell extract as the antigen source. 9/12 (75%) of the pts had detectable a-Ro levels. 3/12 had no detectable a-Ro in spite of multiple exacerbations of skin disease activity. 8/12 (67%) had fluctuations in a-Ro levels during the course of the study. 7 of these 8 pts showed a rising titer after the onset of skin disease activity. 4 of these 7 pts showed a ≥2 titer increase in a-Ro. Because of the insensitivity of the ID assay we are currently re-evaluating these same sera with an ELISA assay using purified Ro/SSA antigen. This sensitive technique will enable us to quantitate levels of individual a-Ro Ig classes in addition to detecting small fluctuations in a-Ro levels. These preliminary results suggest that there is no significant increase in a-Ro ID titers prior to or coincident with flares of skin disease activity in SCLE pts. These findings argue somewhat against the possibility that free a-Ro is directly mediating cutaneous injury in SCLE, but do suggest that elevations of a-Ro levels in these pts might result from increased antigen load released during cutaneous tissue injury.

4. Antibodies to Single-Stranded DNA (ssDNA) in Various Cutaneous Lupus Erythematosus (LE) Subsets. J. P. CALLEN*, K. B. KULICK†, AND C. MACDONALD†, * University of Louisville School of Medicine, Louisville, KY, and † State University of New York, Buffalo, NY.

Antibodies to ssDNA have been found in about 20% of patients with chronic cutaneous (discoid) LE (DLE). Previously we have suggested that the presence of elevated titers of anti-ssDNA correlated with widespread DLE, active cutaneous disease, an elevated ESP and a greater risk of fulfilling the criteria for the diagnosis of systemic LE (SLE). We have measured ssDNA antibodies in 62 patients with cutaneous LE consisting of 22 patients with subacute cutaneous LE (SCLE), 30 patients with DLE and 10 with both SCLE and DLE. Elevated levels of antibodies to ssDNA were found in 13 patients (20.9%). The elevated levels were distributed among the cutaneous subsets as follows: 2 patients had both papulosquamous (PS) SCLE and DLE, 2 patients had PS SCLE, 3 patients had annular SCLE, 4 patients had localized DLE and 2 patients had widespread DLE. Criteria for the diagnosis of SLE were fulfilled in 9 of the 13 patients with elevated ssDNA in contrast to 11 of the 49 with normal levels ($p < 0.001$). Furthermore, elevated ssDNA antibodies were present in 6 of 7 patients with clinical renal disease, but only 7 of the 55 without renal disease ($p < 0.0001$). Seven of the 13 patients with elevated ssDNA had a positive ANA, of whom 4 also had anti-Ro antibodies. No consistent immunogenetic HLA typing was present. Thus, we have shown (1) that antibodies to ssDNA are present in patients with SCLE as well as those with DLE; (2) that elevated levels can be present in patients with DLE localized to the head and neck; and (3) although not specific, the presence of elevated levels of antibodies to ssDNA in the patient with cutaneous LE correlates with multisystem disease (criteria for SLE) and with clinical renal disease.

5. Carcinogen-Induced Chromosome Breakage As a Marker for First Trimester Prenatal Diagnosis and Complementation Analysis in Fanconi Anemia Cells. A. D. AUERBACH, V. S. VENKATRAJ, AND D. M. CARTER, Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY.

Despite absence of knowledge of the specific molecular defect in Fanconi anemia (FA), hypersensitivity of FA cells to the clastogenic effect of DNA crosslinking agents provides a unique marker for the syndrome. We have found that analysis of diepoxybutane (DEB)-induced chromosomal breakage can be used to facilitate first trimester prenatal diagnosis in chorionic villus (CV) cells. CV cells from 5 pregnancies at risk and 4 controls were monitored. In 4 cases results in the at-risk and control cells were similar; the range of spontaneous and DEB-induced breakage was 0–0.10 and 0.03–0.15 breaks/cell respectively. One fetus was diagnosed as affected; mean spontaneous and DEB-induced breakage was 0.46 and 1.4 breaks/cell respectively. This pregnancy was terminated and the diagnosis confirmed by cytogenetic studies in several fetal tissues.

Using hybridoma technology to study genetic complementation in FA, we fused an HGPRT-deficient lymphoblastoid cell line from a male FA patient with normal female peripheral blood lymphocytes. Antibody-secreting hybridoma cells with a chromosomal complement of 92, XXXY were isolated. The hybrid cells showed complementation of DEB-induced breakage, exhibiting a frequency of 0.08 breaks/cell. We feel these techniques will be useful for analysis of genetic heterogeneity and gene dosage effect in FA and similar disorders, and thus further our understanding of the nature of a gene affecting hypersensitivity to environmental clastogens.

6. Effects of a Polyvalent Tumor Antigen Vaccine in Human Malignant Melanoma. J.-C. BYSTRYN, P. BERNSTEIN, M. HARRIS,

D. ROSES, AND J. SPEYER, Depts. of Dermatology, Surgery, and Medicine, NYU School of Medicine, New York, NY.

A polyvalent melanoma antigen vaccine was used to treat 37 patients with malignant melanoma, 20 patients were treated post surgical resection of regional disease (stage II), and 17 patients had disseminated melanoma (stage III). The vaccine was prepared from surface material shed by pooled allogeneic melanoma cells, contained at least 12 different MAAs, and was free of fetal calf serum proteins. It was administered intradermally without adjuvant into all 4 extremities in: a) escalating doses (0.25 to 50 μg/site) weekly $\times$ 8; b) fixed dose (10 μg/site) every 2 wks $\times$ 4; or c) with cyclophosphamide pretreatment (300 mg/m^2 3 days prior to each immunization) every 3 wks $\times$ 4 to inactivate suppressor cells in some patients with disseminated melanoma. Booster doses were subsequently given to all patients monthly $\times$ 3 and every 3 months thereafter. There was no toxicity other than transient erythema at injection sites. Humoral and/or cellular immunity to melanoma increased in 30 (81%) of the patients. Cellular immunity increased more frequently than antibody response, immunization every 2 weeks was more immunogenic than the weekly schedule, and pretreatment with cyclophosphamide appeared to enhance immunogenicity, 85% of 20 patients with stage II melanoma remain disease free after a mean follow-up period of 8 (range 3–21) months. Of the 10 patients with stage III disease who have been followed longer than one year, 1 had a complete remission and has been disease free for 2 years, and one has stable disease with no progression for 14 months. These results indicate that immunization with a polyvalent melanoma antigen vaccine is safe, and increases humoral and/or cellular immunity to melanoma in most patients. The effect on the progression of melanoma is still undetermined.

* **Modification of the Argon Laser to Restrict Nonspecific Dermal Injury.** S. V. TANG, D. BOURGELAIS, I. ITZKAN, K. A. ARNDT, J. M. NOE, R. S. STERN, AND B. A. GILCHREST, Boston, MA.

* **Sequential Development of Human Fetal Skin Transplanted to Nude Mice.** A. T. LANE AND K. H. DAY, Rochester, NY.

* **T-Cell Dependent Mast Cells: A Model to Study the Effect of Phototoxic Agents.** H. W. LIM AND S. I. WASSERMAN, San Diego, CA.

* **A Study of Human Skin Grafts on Congenitally Athymic (Nude) Rats.** A. GILHAR, Z. WOJCIECHOWSKI, AND G. KRUEGER, Salt Lake City, UT.

* **Cytotoxicity and Mutagenicity of 193 and 248 nm Laser Radiation in Mammalian Cells.** H. A. GREEN, J. H. BOLL, I. E. KOCHEVAR, T. DEUTSCH, J. A. PARRISH, AND A. R. OSEROFF, Boston, MA.

* **Human Epidermal Arachidonate Metabolism: An In Vitro Model.** V. DELEO, D. HANSON, AND S. SCHEIDE, New York, NY.

* **Factors Influencing Anthralin Irritancy.** T. KINGSTON AND N. LOWE, Los Angeles, CA.

Saturday, May 4, 1985

1:00 PM–4:00 PM Registration Upper Lobby
8:00 AM–12:00 PM Sheraton Washington Ballroom

JOINT SID/JSID-AFCR/ASCI/AAP PLENARY SESSION

Peter Elias, Lloyd King, and David Norris, Presiding

1. Differential *In Vivo* Binding of Specific Lupus Sera in Human Skin. L. A. LEE, W. L. WESTON, J. O. STEVENS, G. G. KRUEGER, M. EMAM, AND D. A. NORRIS, University of Colorado, Denver, CO, and University of Utah, Salt Lake City, UT.

Antibodies to the SSA/Ro antigen are found in 80% and 98% of patients, respectively, with the papulosquamous lupus syndromes subacute cutaneous lupus (SCLE) and neonatal lupus (NLE). Though SSA antigen has been demonstrated in and on keratinocytes, it is not known if antibodies to this antigen are directly related to the diseases. We have shown in an animal model, the nude mouse with grafted human skin, that intravenous (IV) injection of anti-SSA serum results in granular deposits of IgG in human skin at the dermal-epidermal (D-E) junction and on basal keratinocytes, and that this deposition is augmented by ultraviolet (UV) light. To determine if IgG binding to basal keratinocytes is specific for certain lupus sera in this model, we compared anti-SSA sera from 3 SCLE/NLE patients to sera from 5 SLE patients without skin disease who had comparable or higher titers of antinuclear antibodies, either to native DNA (nDNA) or to Sm. Animals were injected IV with 0.3 cc serum and biopsies were taken 24 hours later. In all 10 animals injected with anti-SSA sera, there was granular IgG on basal keratinocytes in the graft. In none of the 7 animals injected with SLE sera was there deposition of IgG at the epidermis or D-E junction, even after UV light exposure of the graft.

We conclude that there is specific binding of anti-SSA antibodies to SSA antigen *in situ*: (1) IgG from anti-SSA sera was found in human skin, which contains SSA antigen, but not in mouse skin, which does not; (2) binding of IgG to skin did not occur with anti-nDNA or -Sm SLE sera, even after UV light. The IgG deposition with anti-SSA sera occurs on the basal keratinocyte, where cellular injury is seen in histologic exam of lupus lesions, and the pattern of deposition in the animal model reproduces the pattern observed in SCLE and NLE patients' lesions. Specific antibody binding *in situ* to basal keratinocytes may be directly related to the development of SCLE and NLE lesions.

2. Binding and Inhibition of ETAF Activity by Monoclonal Anti-IL1 Antibody. ANDREAS KÖCK, MARTIN DANNER, AND THOMAS A. LUGER, Dept. of Dermatology II, University of Vienna, Vienna, Austria.

In order to generate a monoclonal antibody against Interleukin 1 (IL1), Balb/c mice were immunized with partially purified human peripheral blood lymphocyte derived IL1. After immunization spleen cells were hybridized with a non-secreting plasmocytoma cell line. Hybridoma cell line supernatants were screened for anti IL1 activity by testing their ability to inhibit IL1 mediated thymocyte proliferation as well as by their capacity to bind to IL1 in a dot immunobinding assay. After cloning by limiting dilution several clones with anti IL1 activity were obtained. For ascitic antibody production one clone was selected. Ascitic anti IL1 antibody (IgG2a) blocked IL1 mediated thymocyte and fibroblast proliferation and bound to IL1 even when diluted 1:2500. Similarly the monoclonal anti IL1 antibody inhibited the effects of ETAF on thymocyte and fibroblasts and also bound to ETAF of human as well as a murine origin. Furthermore using monoclonal anti IL1 antibody and fixed S. aureus cells it was possible to immunoprecipitate IL1 as well as ETAF. When IL1 containing supernatants were passaged over an anti IL1 antibody CNBr sepharose column >90% of the activity was removed. Elution of IL1 activity from the column yielded >20% of bound IL1 activity. Anti IL1 antibody did not inhibit the proliferation of macrophage, T cell, B cell or fibroblast cell lines. In contrast to a monoclonal anti IL2 antibody, the anti IL1 antibody, neither inhibited IL2 mediated proliferation of CTLL cell lines nor bound to recombinant IL2. These data indicate that monoclonal anti IL1 may be usefull for the development of new immunoassays for the detection of ETAF/IL1. Furthermore the antibody may provide a useful tool for dissecting the multiplicity of biological effects of IL1/ETAF and may also be helpful for the investigation of the in vivo role of these cytokines.

* **A New Approach for the Diagnosis and Treatment of Psoriasis.** M. F. HOLICK, J. A. MACLAUGHLIN, W. GANGE, AND E. SMITH, Boston, MA.

3. The Genes for Epidermal Keratins and Other Intermediate Filaments Arose from a Common Ancestor. P. STEINERT, T. KRIEG, L. JOHNSON, W. IDLER, AND D. ROOP, National Cancer Institute, NIH, Bethesda, MD.

Differentiated mammalian cells contain a class of cytoplasmic proteins known as intermediate filaments (IF). In epithelial cells they are known as keratin IF. All IF consist of a heterogeneous group of subunits and are built according to a common plan: a central α-helical domain of conserved size and secondary structure, flanked by non-α-helical end domains of variable size and amino acid sequence. There are 3 known types of α-helical sequences in IF subunits: acidic keratins form type I; basic keratins form type II; and vimentin, desmin and GFA

* Asterisks indicate abstracts submitted by AFCR/ASCI/AAP and published in *Clinical Research*.

form type III. In order to examine the evolutionary relationships between IF subunits, we have studied 2 keratin genes, which encode the type I mouse 59 kD keratin and the type II human 67 kD keratin. These genes were isolated from genomic libraries by their hybridization to specific cDNAs. They occur only once in the haploid genome of their species. They were characterized by R-loop and sequence analyses to identify the numbers and locations of introns. Comparisons of their structures with that published for the hamster type III vimentin gene show several similarities. Each gene has 5 or 6 introns in the regions which encode the α-helical domain, but none corresponds to any known structural subdomain. Interestingly, the positions of 4 introns in the α-helical domain have been conserved among the 3 types of genes, and almost all introns splice at the first amino acid residue of the characteristic heptad repeat of the α-helical domain. These data suggest that the 3 types of IF genes share a common ancestor which may have been assembled from smaller units containing multiple heptad repeats. Subsequent evolutionary duplication events may then have formed the 3 known α-helical types and each of its various members.

* **Human Alveolar Macrophages Produce a Fibroblast-like Collagenase and Collagenase Inhibitor.** H. G. WELGUS, E. J. CAMPBELL, Z. BAR-SHAVIT, R. M. SENIOR, AND S. L. TEITELBAUM, St. Louis, MO.

* **Effect of Ultraviolet Radiation on Antigen Presentation.** S. SHIMADA, Z. KOVAC, R. H. SCHWARTZ, AND S. I. KATZ, Bethesda, MD.

* **Ultraviolet B (UVB) Radiation Impairs Human Natural Killer (NK) Cell Activity.** C. A. ELMETS, K. LARSON, G. A. URDA, AND B. Z. SCHACTER, Cleveland, OH.

4. **Novel Immunotherapy of Melanoma Using Cytotoxic Lymphokine-Producing Autoreactive Cloned Helper T Cells.** TETSUO SHIOHARA, KIRSTEN A. JACOBSON, AARON B. LERNER, MARK HOROWITZ, AND NANCY H. RUDDLE, Depts. of Dermatology, Pathology, and Epidemiology, Yale University School of Medicine, New Haven, CT.

The identity of the principal effector lymphocyte in tumor rejection *in vivo* is controversial. Whereas formerly Lyt-2$^+$ cytolytic T cells were thought to be the main candidates, recent studies suggest that Lyt-1$^+$ T cells may play an important role. Seeking T-cells highly effective in adoptive immunotherapy for melanoma, we have established and characterized several murine Lyt-1$^+$ cloned helper T cells with lytic activity against a variety of tumor cells. We found that self-Ia-restricted autoreactive cloned T cells, derived from C57BL/6J mice, lysed self-Ia$^+$ target cells *in vitro*. In the presence of self-Ia antigen or Con A they also lysed Ia$^-$ bystander targets such as Cloudman and B16 melanoma and lymphoid tumor cells. Activation of the autoreactive T cells by self Ia antigen or Con A resulted in the production of at least two cytotoxic lymphokines, i.e., lymphotoxin and IFN-γ. In fact, the supernatants were cytostatic to B16 melanoma cells *in vitro*. To assess the *in vivo* anti-tumor activity of the autoreactive T cells, different numbers of these cells ($1–5 \times 10^6$) were injected simultaneously with 1×10^6 B16 melanoma cells into the ear pinnae of sublethally irradiated syngeneic mice. Even at the lowest dose (1×10^6 cells) the autoreactive T cells inhibited the growth of tumors. A different Ia-restricted Lyt-1$^+$ T cell clone, one that produces IFN-γ but not lymphotoxin in response to Ia *plus* soluble antigen, was not effective against B16 melanomas *in vivo* in the absence of activation by soluble antigen. We interpret these results to indicate that Ia-restricted Lyt-1$^+$ cells after activation with specific antigen *in vitro* and *in vivo* can inhibit bystander B16 melanomas via release of cytotoxic lymphokines. These findings suggest a novel approach to adoptive immunotherapy of melanoma.

* **Bullous Pemphigoid Sera Contain Antibodies to Placental Type V Collagen Chains.** D. T. WOODLEY, M. J. REESE, T. YOSHIIKE, E. J. KEEFE, R. A. BRIGGAMAN, AND W. R. GAMMON, Chapel Hill, NC.

5. **Immunofluorescent Localization of the Human Skin Chymotrypsin-like Proteinase to Skin Mast Cells.** NORMAN M. SCHECHTER, JOHN K. CHOI, SHIGETOSHI SAYAMA, DIANE T. DERESIENSKI, AND GERALD S. LAZARUS, Dept. of Dermatology, University of Pennsylvania, Philadelphia, PA.

Human skin contains a chymotrypsin-like proteinase which is elevated in skin lesions of patients with cutaneous mastocytosis. To identify the proteinase directly as a mast cell constituent, immunocytological studies were undertaken. The proteinase was purified from skin using a soybean trypsin inhibitor (SBTI) affinity column and an antiserum was produced in a rabbit. The antiserum did not cross-react with human PMN cathepsin G, human PMN elastase, or human skin tryptase. Indirect immunofluorescent staining of 4 micron frozen skin sections showed distinct cytoplasmic staining of a small population of dermal cells. These cells were scattered throughout the papillary dermis and were particularly noticeable surrounding blood vessels and appendages, places where mast cells are normally distributed. Restaining these sections with toluidine blue showed that the fluorescently stained cells corresponded exclusively to cells containing metachromatically staining granules, the major distinguishing feature of mast cells. Immune IgG absorbed against proteinase purified through both an SBTI and a D-tryptophan methyl ester affinity column showed no specific fluorescent staining. This result verifies the specificity of the antiserum. Human lung is another tissue which contains mast cells. So far only a trypsin-like proteinase has been clearly established as a constituent of these mast cells. Using our antiserum, positive immunofluorescent staining of lung mast cells could be demonstrated. These data demonstrate that the major chymotrypsin-like proteinase of human skin is a constituent of both skin and lung mast cells.

* **Detection and Purification of Histone Hydrolyzing Enzyme in Rat Epidermis.** R. J. HARVIMA, K. YABE, M. NAKAGAWA, J. E. FRAKI, K. FUKUYAMA, AND W. L. EPSTEIN, San Francisco, CA, and Kuopio, Finland.

6. **Epidermal Langerhans Cells Behave as Immature or Precursor Elements in the Dendritic Cell Family.** GEROLD SCHULER, MARGIT WITMER, NIKOLAUS ROMANI, AND RALPH STEINMAN, Laboratory of Cellular Physiology and Immunology, The Rockefeller University, New York, NY, and Dept. of Dermatology, University of Innsbruck, Innsbruck, Austria.

To establish if epidermal Langerhans cells (LC) are related to spleen dendritic cells (DC) we have studied the morphology, phenotype and function of both cell types in culture. LC could be partially enriched (up to 60%) on the basis of 2 simple physical properties: nonadherence and low buoyant density in dense albumin columns. Upon culture LC became very similar to spleen DC. LC were nonadherent, did not proliferate, developed many processes and/or veils and lost their Birbeck granules. Nonspecific esterase and membrane ATP'ase as well as F4/80 (α-macrophage) and 2.4G2 (α-Fc receptor) antigens disappeared, while Mac1 and Ia antigens persisted. Freshly isolated LC surprisingly were very weak T cell stimulators (primary mixed leukocyte reaction, oxidative mitogenesis), but activity per Ia$^+$ cell increased 30-fold in culture—even after irradiation—so that by 2–3 days LC were 3–10 times more potent than spleen DC. Fresh LC were Ia-rich, but in contrast to cultured LC and spleen DC they did not form clusters with responding T cells. Exogenous gamma interferon increased expression of Ia on macrophages but not on DC or cultured LC, nor did it enhance the stimulatory activity of cultured LC.

Our data suggest that LC in situ are precursors of lymphoid dendritic cells and are immunologically immature.

* **Modulations in Lipid Metabolism and Content During Differentiation of Cultured Human Keratinocytes.** M. L. WILLIAMS, T. L. WHITE, B. E. BROWN, M. HINCENBERGS, AND P. M. ELIAS, San Francisco, CA.

7. **Fibroblast Collagen Production Inhibitory Lymphokine and Monokine Are Interferons γ and β Respectively.** MATTHEW R. DUNCAN AND BRIAN BERMAN, Depts. of Dermatology, University of California, Davis, and VA Medical Center, Martinez, CA.

We have previously shown that human peripheral blood mononuclear cells activated with concanavalin A (Con A) or lipopolysaccharide (LPS) produce respectively, lymphokines (LK) of 50,000 Mr, or monokines (MK) of 20,000 Mr that inhibit the growth and collagen production of cultured human dermal fibroblasts. Since these Mr fractions and LK/MK supernates contained anti-viral activity equivalent to 10^2–10^3 U/ml of interferon (IFN) we investigated if any of the fibroblast-inhibitory activites could be attributed to human IFNs. Concentrations of $10–10^5$ U/ml of purified naturally-derived α, β and γ-IFN and recombinant DNA-derived α_2 and γ-IFN did not inhibit the growth of subconfluent foreskin or adult dermal fibroblasts as assessed by ^{3}H-thymidine incorporation into DNA. In contrast, all tested IFNs caused

concentration dependent inhibitions of collagen production by confluent fibroblast microcultures as assessed by ^{3}H-proline incorporation into pepsin-resistant salt-precipitated collagen. At $\geq 10^3$ U/ml γ-IFNs caused maximum inhibitions of 70%–80% and α and β-IFNs maximally inhibited by 50%–60%. Non-collagen protein synthesis was minimally affected. The collagen production inhibitory activity of Con A-induced LK supernate and its 50,000 Mr fraction was completely suppressed by 10^3 neutralizing U/ml of either polyclonal or monoclonal antibody to γ-IFN, while polyclonal antibodies to α and β-IFN had no effect. Similarly, the collagen production inhibitory activity of LPS-induced MK supernate and its 20,000 Mr fraction was suppressed by polyclonal anti-β-IFN but not by anti-α or anti-γ-IFN. Anti-IFNs failed to reverse LK/MK growth-inhibitory activities. These data suggest collagen production inhibitory LK and MK are γ-IFN and β-IFN respectively and that growth inhibitory LK/MK are not IFNs.

* **Molecular Heterogeneity of the Bullous Pemphigoid Antigens Detected by Immunoblotting.** R. S. LABIB, G. J. ANHALT, H. P. PATEL, AND L. A. DIAZ, Baltimore, MD.

* **Induction of Collagenase Expression in Human Fetal Skin Fibroblasts and Certain Other Human Cells by a Phorbol Diester.** S. M. WILHELM AND A. Z. EISEN, St. Louis, MO.

8:00 AM–12:00 PM Sheraton Washington Marshall Room

AFCR/ASCI/AAP DERMATOLOGY SESSION

* **Non-invasive Measurements of Skin and Cornea Using Femtosecond Laser Pulse Optical Ranging.** A. R. OSEROFF, J. FUJIMOTO, S. DESILVESTRI, C. A. PULIAFITO, R. MARGOLIS, AND J. A. PARRISH, Boston, MA, and Cambridge, MA.

* **Phagocytosis of Keratin Filament Aggregates by Human Monocytes and Polymorphonuclear Neutrophils.** H. HINTNER, N. ROMANI, U. STANZL, P. FRITSCH, AND T. J. LAWLEY, Bethesda, MD.

* **Epidermal Cells Produce Mediators Capable of Regulating Fibroblast Functions.** A. D. HERNANDEZ, G. P. STRICKLIN, A. E. POSTLETHWAITE, E. A EVERS, AND M. A. CATES, Memphis, TN.

* **Modulation of Collagenase Production by Human Monocytes in Culture.** AKIHIDE OHTA, J. S. LOUIE, L. RYHANEN, AND J. UITTO, Torrance, CA.

* **Rat-Mouse Hybridomas Producing Monoclonal Antibodies Reactive with a Basal Cell Hemidesmosome Antigen Similarly to Human Bullous Pemphigoid Autoantibodies.** C. L. BUREK, E. AVALOS, N. R. ROSE, D. F. MUTASIM, R. S. LABIB, H. P. PATEL, G. J. ANHALT, AND L. A. DIAZ, Baltimore, MD.

* **Nidogen and Heparan Sulfate Proteoglycan: Detection of Newly Isolated Basement Membrane Components in Normal and Epidermolysis Bullosa (EB) Skin.** S. W. CAUGHMAN, T. KRIEG, H. HINTNER, R. TIMPL, S. I. KATZ, Bethesda, MD, Munich, F.R.G., and Innsbruck, Austria.

* **Peanut Agglutinin Defines an Epidermal Cell Membrane Defect in Skin from Patients with Epidermolysis Bullosa Simplex.** JO-DAVID FINE, Birmingham, AL.

* **Defining the Role of Complement in Experimental Pemphigus Vulgaris in Mice.** N. F. EAGLSTEIN, G. TILL, L. A. DIAZ, R. S. LABIB, H. P. PATEL, AND G. J. ANHALT, Baltimore, MD, and Ann Arbor, MI.

* **Comparison of Plasminogen Activator Localization in a Pemphigus Lesion and in Psoriatic Plaque.** S. MORIOKA, P. J. JENSEN, AND G. S. LAZARUS, Philadelphia, PA.

* **Brazilian Pemphigus Foliaceus Patients Have Elevated Thymosin Alpha-1 Levels.** J. T. ROSCOE, P. H. NAYLOR, L. A. DIAZ, R. S. LABIB, H. P. PATEL, A. D. GOLDSTEIN, S. A. P. SAMPAIO, AND G. J. ANHALT, Baltimore, MD, Washington, DC, and Sao Paulo, Brazil.

* **Phototoxicity in Mast-Cell Deficient, C5-Deficient and Leukopenic Mice.** H. W. LIM, M. HAGAN, AND I. GIGLI, San Diego, CA.

* **Local Ultraviolet Radiation Enhances the Elicitation of Contact Hypersensitivity in the Mouse.** L. POLLA, C. GOULSTON, J. A. PARRISH, AND R. D. GRANSTEIN, Boston, MA.

* **Freshly Isolated Mouse Epidermal Cells (EC) Enriched for Thy+ Cells Fail to Exhibit Natural Killer (NK)-like Activity.** J. L. NIXON-FULTON, P. R. BERGSTERSSER, J. HACKETT, JR., V. KUMAR, AND R. E. TIGELAAR, Dallas, TX.

* **Inhibition of Neutrophil Chemotaxis by 13-cis-Retinoic Acid May Be Endothelial Cell Dependent.** M. G. TONNESEN, R. L. OSBORN, AND D. A. NORRIS, Denver, CO.

* **Therapy for Brown Recluse Spider Bites Is Dependent on Venom Persistence.** R. S. REES AND L. E. KING, JR., Nashville, TN.

* **Polymorphic Analysis of the Human Keratin Genome.** A. S. VAN VOORHEES, I. M. FREEDBERG, AND M. BLUMENBERG, New York, NY.

12:00 PM–1:00 PM Sheraton Washington Exhibit Hall

AFCR/ASCI/AAP DERMATOLOGY POSTER SESSION

* **A Model for Measuring Microcirculation in Skin.** S. E. HUETHER, Z. J. WOJCIECHOWSKI, T. J. PETELENZ, L. G. LEONARD, AND G. G. KRUEGER, Salt Lake City, UT.

* **Psoralen-Induced Skin Photosensitization by Reactive Oxygen Species ($^1O_2$, O_2 and OH).** M. A. PATHAK, P. C. JOSHI, AND Z. ZAREBSKA, Boston, MA.

* **IgA Circulating Immune Complexes Occur in the Sera of Patients with Dermatitis Herpetiformis in the Absence of IgA Rheumatoid Factor.** R. P. HALL AND R. W. EYRE, Durham, NC.

* **Erythema Nodosum Leprosum (ENL) Is a Loss of a State of Immune Suppression in Lepromatous Leprosy (LL).** R. L. MODLIN, K. UYEMURA, L. WONG, AND T. H. REA, Los Angeles, CA.

* **Effect of Retinoid on Neutrophils.** A. R. SHALITA AND W. L. LEE, Brooklyn, NY.

* **Blood Eosinophilia and Anti-ssDNA Antibodies in Linear Scleroderma.** V. FALANGA, T. A. MEDSGER, JR., AND M. REICHLIN, Oklahoma City, OK.

* **Phenotypic Differences Between Epithelial Keratinocytes of Human Conjunctiva and Cornea.** R. M. MCCALLUM, T. J. PALKER, L. M. COBO, AND B. F. HAYNES, Durham, NC.

* **Correlation Between Keratinocyte Expression of Ia and the Intensity of a Contact Hypersensitivity Response.** L. K. ROBERTS, G. J. SPANGRUDE, R. A. DAYNES, AND G. G. KRUEGER, Salt Lake City, UT.

* **Pemphigus and Pemphigoid Antigen Expression in Two Cultured Human Keratinocyte Lines.** D. CHERNOSKY, K. A. FRITZ, A. M. BEYER, AND R. E. JORDAN, Houston, TX.

* **Cutaneous Vascular Sensitivity to Lower Aliphatic Alcohols and Aldehydes in Oriental, Alcohol Flushers.** J. K. WILKIN AND G. W. FORTNER, Richmond, VA.

1:30 PM–2:15 PM Presidential Ballroom

JSID GUEST LECTURER

Shiego Nishiyama, Presiding

Akira Kobata

"The Sugar Chains of Membrane Glycoproteins—Structural Changes Induced by Malignant Transformation and Their Application for the Diagnosis of Cancer"

2:30 PM–5:30 PM

CONCURRENT SCIENTIFIC SESSIONS

CONCURRENT SESSION 4A: PHOTOIMMUNOLOGY

Congressional

Kiichiro Danno and Craig Elmets, Presiding

1. Ultraviolet Light-Irradiated Langerhans Cells Fail to Stimulate Allo-Ia-Reactive T Cells to Produce T Cell Differentiation Factor. T. TSUCHIDA, J-H. QIAN, H. IZUMI, H. FUJIWARA, AND T. HAMAOKA, Institute for Cancer Research, Osaka University Medical School, Osaka, Japan.

The effect of ultraviolet light radiation (UVR) on the alloantigen-presenting capacity of Langerhans cells (LC) in the in vitro cytotoxic T lymphocyte (CTL) response was analyzed. Allogeneic murine epidermal cells (EC) could potently stimulate nylon wool-purified T lymphocytes, whereas UVR-EC (UVB dose: 1000 J/m^2) could not stimulate. Fluorescent staining of UVR-EC by various monoclonal anti-Ia antibodies revealed no significant reduction in Ia-reactivity. Moreover, the addition of UVR-EC did not inhibit the CTL response stimulated by EC, excluding the possible active suppression mechanism. The bypassing of helper T lymphocyte requirement by Concanavalin A-activated spleen cell supernatant (containing IL-2 and other factors) could correct the UVR-induced impairment of CTL response, whereas purified recombinant IL-2 alone could not correct. This result clarified two points; 1) class I MHC molecules recognized by CTL are not impaired by UVR, and 2) the additional factor other than IL-2 is required for the correction of UVR-induced impairment of CTL response. The additional factor could be provided by the supernatant of mixed lymphocyte-EC culture. Interestingly, this supernatant contained no detectable IL-2 activity but high CTL-differentiation-inducing activity measured by the differentiation of thymocytes into CTL. In contrast, the supernatant of T lymphocyte culture stimulated by UVR-EC revealed very low CTL-differentiation-inducing activity. Thus, the UVR-LC apparently lost alloantigen-presenting activity of Ia molecule to produce CTL-differentiation factor, but this impairment could not be detected by the loss of reactivity to a certain monoclonal anti-Ia antibody so far tested.

2. Effect of Ultraviolet Radiation on Ia Expression by the Epidermis. A. GILHAR, L. K. ROBERTS, R. A. DAYNES, AND G. G. KRUEGER, Depts. of Internal Medicine (Dermatology) and Pathology, University of Utah School of Medicine, Salt Lake City, UT.

Some skin diseases are characterized by both the infiltration of Ia+ lymphocytes and the expression of Ia by the keratinocytes. Since ultraviolet radiation (UVR) has a therapeutic effect on these diseases, the present study was undertaken to determine whether UVR exerts its effect through altering keratinocyte expression of Ia. To test this, nude mice with one ear protected by electrical tape were exposed to UVR (5,500 J/m^2/day) and then given an injection of normal mouse serum to induce keratinocyte expression of Ia. UVR treated mice injected with nude mouse serum and non-UVR exposed animals injected with normal mouse serum served as Ia− and Ia+ controls respectively. Analysis of Ia expression was made by indirect immunoperoxidase staining using monoclonal antibodies specific for I-A and I-E determinants. Exposures (4 days for 30 min) led to LC depletion by marker analysis in nonprotected ears, with no effect on the protected ears. In a second experiment, after 4 UVR treatments the animals were injected with the appropriate serum and continued on UVR exposures (5 days for 15 min). On day 10, epidermal sheets were analyzed for Ia expression. Results showed that keratinocytes were induced to express Ia in UVR protected ears and non-UVR treated mice injected with normal mouse serum. In contrast, the keratintocytes in the UVR nonprotected ears were Ia-. These results demonstrate that the inhibition of Ia expression in the epidermis by UVR is a local phenomenon and is consistent with the notion that UVR may alter disease processes by exerting an effect on Ia expression.

3. cis-Urocanate, a UV Irradiation Product, Decreases the Number of Ia-Positive Langerhans Cells in Cultured Epidermal Sheets. F. P. NOONAN, E. C. DE FABO, AND H. MORRISON, School of Medicine, Flinders University of South Australia, Bedford Park, South Australia, George Washington University, Washington, DC, and Purdue University, Lafayette, IN.

Ultraviolet (UV) irradiation of mice (wavelengths 250–320 nm) causes an immune suppression. Contact hypersensitivity (CHS) is decreased if sensitiser is applied to an unirradiated site, and there is a defect in antigen presenting cells (APC). Our previous studies suggested that UV-induced suppression of CHS is initiated by an interaction between UV and urocanic acid (UCA) in the stratum corneum. With UV, UCA photoisomerises from the natural trans form to the cis isomer. We have tested the hypothesis that cis UCA modulates Ia expression on APC.

EDTA-separated epidermal sheets were cultured at 37C in medium with or without trans or cis UCA (prepared by HPLC). Ia-positive cells (LC) were determined by a FITC linked or a peroxidase linked stain using mouse monoclonal anti-IA antibodies. LC were also enumerated by an ATPase or a β-glucuronidase stain. After 3 days of culture, the number of cells detected by all methods was unaltered in control cultures or in cultures with trans UCA. In cultures with cis UCA, the number of IA-positive cells was significantly decreased by 25–35% ($p < 0.001$), but the number of ATPase positive and of β-glucuronidase positive cells was unaffected. Concentrations of 20–200 μg/ml of cis UCA were effective on epidermal sheets from Balb/c mice (IA^d), or C$_3$H mice (IA^k). The number of IA positive cells was unchanged in epidermal sheets incubated with cis UCA at 4C. Addition of cis UCA to anti-IA antibody did not alter staining. In contrast, incubation with cis or trans UCA (50 μg/ml) did not alter Ia induction by a Con A supernatant on a mouse macrophage cell line PU-5-IR. These findings are consistent with an effect of cis UCA on Ia expression of a subset of APC.

4. The Effect of UVB, UVC and Temperature Elevation to 42° on the Production of ETAF. THOMAS KUPPER, JOSEPH MCGUIRE, AND LUANN LIGHTFOOT, Depts. of Dermatology and Surgery, Yale University School of Medicine, New Haven, CT.

The effect of UVB, UVC and temperature elevation to 42° on the production of ETAF has been examined in the following cultured cells: Neonatal human foreskin keratinocytes (HFEC) in secondary cultures, a transformed mouse keratinocyte (PAM), and a human epidermal carcinoma (A431). ETAF was measured by determining ^{3}Htdr incorporation into D10.G4.1 (a cloned T helper cell) in the presence of concanavalin A. Proliferation of this clone is IL-1 dependent.

UV254 nm caused increased production of ETAF by PAM and had little effect on A431 which has high constitutive levels. Constitutive levels of ETAF in HFEC were low and only slightly stimulated by UV254 nm. UV300 nm caused increased production of ETAF in PAM cells had little effect on HFEC or on A431 which had high preirradiation levels of ETAF. Keratinocytes freshly isolated from neonatal foreskin produced increased amounts of ETAF at 42°.

Elevation of temperature from 37° to 42° for 4 hours resulted in substantial stimulation of ETAF production in PAM and HFEC. Temperature elevation did not stimulate ETAF production by A431 which spontaneously produces large amounts of ETAF.

The increase in ETAF caused by temperature elevation represents a potentially important interaction between hyperthermia and immunoregulation since others have shown that T cell proliferation in response to IL-1 is sensitive to small elevations in temperature.

5. Suppression of Multiple Hapten-Specific Immune Responses by UV-Induced Suppressor Cells. STEPHEN E. ULLRICH, GENE K. YEE, AND MARGARET L. KRIPKE, University of Texas System Cancer Center, Dept. of Immunology, M. D. Anderson Hospital, Houston, TX.

Exposure of mice to ultraviolet-B (UV) radiation interferes with their ability to respond to a contact allergen applied epicutaneously at a distant unexposed site. This immunosuppression is associated with the appearance of hapten-specific suppressor T lymphocytes, and the suppression of contact hypersensitivity (CHS) can be transferred to a nonirradiated recipient by the adoptive transfer of these lymphocytes. We wished to determined whether other immune responses besides CHS were regulated by these UV-induced suppressor cells. In addition to suppressing CHS, the cells from UV irradiated and hapten sensitized animals suppressed the primary lymphoproliferative response of T cells to hapten modified splenic adherent cells, as well as the generation of cytotoxic T cells to hapten-modified self. Only primary immune responses were affected; no suppression was noted when the responder

cells were obtained from a pre-sensitized animal. In addition, introduction of the preformed suppressor lymphocytes into unirradiated recipients rendered them incapable of generating an antibody response against haptenated erythrocytes. These data demonstrate that hapten specific, UV-induced suppressor cells are capable of regulating a variety of hapten-specific immune responses. Whether the combination of UV-irradiation followed by hapten sensitization induces one suppressor cell that regulates antibody production, generation of cytotoxic lymphocytes and lymphoproliferation or induces multiple subsets of regulatory lymphocytes remains to be determined.

6. Suppression of Graft-versus-Host Reactivity by Ultraviolet Radiation. WARWICK L. MORISON AND RICHARD A. PIKE, NCI-Frederick Cancer Research Facility, Frederick, MD.

We have investigated the mechanism of UVB-induced suppression of the local graft-versus-host (GVH) reaction. We first established that a single exposure of recipient (C57BL6 × C3H$^-$) F_1 (B6C3F_1) mice to UVB radiation suppressed the GVH reaction to parental C3H^{-1} lymphoid cells, as measured by the popliteal lymph node weight gain assay. Several observations provided evidence to suggest that this effect of UVB radiation is nonspecific and involves an alteration of the host lymphoid cell component of the reaction. First, the nonspecific trauma of mild thermal injury also suppressed the GVH reaction. Second, although treatment of mice with rose bengal and visible radiation suppresses contact hypersensitivity while treatment with eosin and visible radiation does not, both types of phototoxic treatment suppressed the GVH reaction. Third, implantation of spleens from normal B6C3F_1 mice into UVB-treated or thermally injured recipient mice, at the time of injection of graft cells, overcame the suppression of the GVH reaction. Finally, treatment of donor B6C3F_1 mice with UVB radiation did not suppress the host-versus-graft reaction in recipient C3H$^-$ mice, which suggests that radiation does not alter the stimulatory function of B6C3F_1 cells. These findings are all consistent with a hypothesis that UVB radiation suppresses GVH reactivity by reducing the host component of this immune response through diversion cells from the site of the reaction. Thus, an alteration of cell trafficking appears to be an additional pathway by which UVB radiation can produce immunosuppression.

7. Mediators of SSA, RnP and Sm Antigen Expression on Human Keratinocyte Surfaces. S. K. JONES, R. M. KISSINGER, L. A. LEE, AND D. A. NORRIS, Dept. of Dermatology, University of Colorado School of Medicine, Denver, CO.

Specific antinuclear antibodies are useful in identifying subsets of lupus erythematosus (LE) and may be directly involved in the aetiology of cutaneous lupus lesions. UV irradiation has been shown to induce (*in vitro*) expression of SSA, RnP & Sm antigens on keratinocyte cell surfaces. Further studies have shown that SSA, RnP, & Sm antibodies would stimulate lysis by mononuclear cells of targets coated with the relevant antigens, linking antibody with keratinocyte damage in LE lesions. This is supported by the observation that human skin grafted into nude mice binds SSA antibodies *in vivo*, this being increased by UV irradiation. The present study was undertaken to determine whether other immunologic, metabolic or cell injury stimuli might induce keratinocyte cell membrane expression of nuclear antigens *in vitro*.

Human keratinocyte cultures were grown in MCDB 153 on Lab-Tek slides. Test substances include δ interferon (10, 100 & 500 μ/ml), cholera toxin (2.5 nM), phorbol-122-myristate-13-acetate (PMA) (1 & 10 ng/ml) appied for 12, 24, 48 and 72 hours and hydrogen peroxide (H_2O_2) (10^{-7} & 10^{-8} M) applied for 2 hours with 24 hours subsequent culture in MCDB. Four slides were used for each experiment. After incubation, one was assessed for viability (acridine orange/ethidium bromide), the other three being incubated with human sera containing a) anti-SSA antibodies, b) anti-RnP antibodies and c) no nuclear antibodies. Binding of human IgG antibodies to the surface of keratinocytes was assessed by immunofluorescence.

None of these stimuli induced cell surface antigen expression, thus supporting the hypothesis that it is specifically UV light rather than T cell specific immune (δ IF), metabolic (chloera toxin), cell injury (H_2O_2), or non-specific (PMA) stimuli, which induces antigen expression on the keratinocyte. This may be a critical step in antibody dependent keratinocyte damage seen in LE.

8. A Sensitive and Specific Assay to Detect and Quantitate Ro(SSA) and La(SSB) Antibodies. RAFAEL HERRERA-ESPARZA, HANY Y. HALIM, THOMAS T. PROVOST, AND LUIS A. DIAZ, Johns Hopkins Medical Institutions, Baltimore, MD.

Ro (SSA) and La (SSB) determinations are important in the evaluation of cutaneous lupus erythematosus patients. In order to quantitate these antibody systems, we developed an assay using partially purified Ro (SSA) and La (SSB) antigens and immunoblot techniques. Fresh, human spleens obtained at autopsy were homogenized in a buffer containing 1.5 mM of phenylmethylsulfonyl fluoride (PMSF). The homogenate was dialyzed and applied to a DEAE column. The active fractions were further purified by a Sephacryl S-300 column. The Ro (SSA) and La (SSB) antigens eluted in a single peak. SDS-PAGE of this peak was performed and the proteins transferred to nitrocellulose paper. After washing, the nitrocellulose strip was developed with a peroxidase-antiperoxidase technique using peroxidase-labelled anti IgG.

Antigen detection, employing gel double diffusion and the immunoblot technique revealed that Ro (SSA) antisera detected a 58K band while the La (SSB) antisera detected two bands, 40 and 42 Kd. Normal sera failed to detect any proteins. High dilutions (~1 × 10^6) of Ro (SSA) and La (SSB) sera were found to react with the Ro (SSA) and La (SSB) antigens blotted onto the nitrocellulose strips.

The similarities of the immunochemical properties of Ro (SSA) and La (SSB) antigens detected with our techniques compared with those of other laboratories indicate: 1) partially purified spleen extracts are an excellent source of Ro (SSA) and La (SSB) antigens; 2) these antigens can be employed with immunoblotting techniques to provide a sensitive assay which provides molecular weight data of the antigens as well as titers of the respective antibodies.

9. The Inhibitory Effect of PUVA on the Immunity of Experimental Dermatophytosis in Guinea Pigs. TAKESHI HORIO AND TAKASHI OKURA, Dept. of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Experimental dermatophytosis in guinea pigs regresses spontaneously in a few weeks acquiring delayed-type hypersensitivity. We examined the effect of topical PUVA on the disease course and immunity of experimental dermatophytosis which was induced by the inoculation of spores of Trichophyton mentagrophytes. Guinea pigs which had been inoculated on the non-treated skin showed mild erythematous lesions with scaling in a few days and then developed the most intense reaction between 10th and 14th day, revealing oozing and ulceration. The lesion resolved completely to leave an alopecic scar by the third week. On the other hand, animals which had been inoculated on the PUVA-treated site showed only mild squamous, erythematous lesions until 4th postinfective week, when the intense reaction began to appear. Complete regression was observed by the fifth week in these animals. Trichophytin tests performed on the 14th postinfective day were positive in the guinea pigs of non-treated group, while negative in the PUVA-treated animals. The latter group revealed a positive reaction on the fifth week. Culturing revealed that dermatophytes disappeared earlier from the inoculation site in the non-PUVA group than in the PUVA-treated guinea pigs. Reinoculation was eliminated by 2 weeks in both groups. PUVA did not show inhibitory effect on immunity of guinea pigs sensitized by intracutaneous injection of trichophytin. These results indicate that PUVA treatment suppresses the immunity of dermatophytosis and delays the spontaneous resolution of the lesions, and suggest that Langerhans cell is involved in the development of cell-mediated immunity in the experimental dermatophytosis in guinea pigs.

10. PUVA Radiation Suppresses Mast Cell Degranulation Induced by Compound 48/80 or Concanavalin-A. KIICHIRO DANNO, KENICHI TODA, TAKAO TACHIBANA, AND TAKESHI HORIO, Dept. of Dermatology, Kyoto University, Kyoto, Japan.

PUVA is a successful treatment modality of solar urticaria and urticaria pigmentosa. This animal study was performed to investigate the effect of PUVA on immediate-type skin reactions. Ears of ICR strain female mice were treated with topical PUVA (0.5% 8-methoxypsoralen (8-MOP) plus 1.5–2.5 J/sq cm of UV-A light). PUVA in this energy dose range did not cause measurable ear swelling response (ESR) or mast cell (MC) degranulation. At several time intervals after PUVA radiation (2 hr–14 days), ESR to intradermal injection with (1) compound 48/80 (C 48/80) (2.5 mg/mL, 10 μL), (2) concavalin-A (Con-A) (2.0 mg/mL), or (3) a mixture of 5-hydroxytryptamine (5-HT) and histamine (H) as vasodilator (1 mg/mL and 50 mM, respectively) was measured with the aid of a dial thickness gauge using different groups of mice (10 mice each), respectively. Rate of MC degranulation and

MC numbers were also assessed by light microscopy using toluidine blue-stained semithin sections. ESR induced either by C 48/80 or Con-A was significantly suppressed dose-dependently (>42% inhibition) by PUVA between 2 hr–3 days' postirradiation as compared with that in non-irradiated control mice (20 mice); ESR returned to normal levels by 7–14 days. % MC degranulation was remarkably decreased (>48% inhibition) in accordance with a suppressed ESR. Either 8-MOP or UV-A light showed no such effects. There were no changes in MC numbers. 5-HT/H-mediated ESR was not significantly altered by PUVA, suggesting that vascular responses to chemical mediators were not affected by PUVA. It seemed therefore that decreased skin reactivity to MC liberators may be due to a PUVA-induced noncytolytic alteration in MC release mechanisms.

11. Dose Response Studies for UVA in the Induction and Elicitation of Contact Photosensitivity in BALB/c Mice. G. M. SHIVJI, W. R. BROWN, AND C. A. RAMSAY, University of Toronto, Toronto, Ontario, Canada.

Contact photosensitivity (CPS) that has been induced in an experimental animal may not be detected if the elicitation dose of UVA is too low. We performed UVA dose response studies for the induction and elicitation of CPS to tetrachlorsalicylanilide (TCSA) in BALB/c mice. Groups of mice were photosensitized on days 1 and 2 by the application of 50 μl of 1% TCSA in acetone to the clipped dorsal skin followed by varying UVA doses (45 mJ/cm^2 to 2.16 J/cm^2). On day 8 elicitation was performed with 10 μl of 0.5% TCSA in acetone to each ear followed by varying doses of UVA (180 mJ/cm^2 to 2.16 J/cm^2). Increase in ear thickness was measured 24 and 48 hours after elicitation. The challenge procedure in non sensitized animals produced an increase in ear thickness of 1.75 ± 0.89 (S.D.) × 10^{-3} cm. Sensitizing doses of UVA of 180 mJ/cm^2 or more produced maximum ear swelling 24 hours after elicitation. At sensitization doses less than 180 mJ/cm^2 the maximum response was delayed to 48 hours. With a sensitizing dose of 180 mJ/cm^2, an elicitation dose of 540 mJ/cm^2 produced a mean increase in ear swelling of 4.5 ± 5.7 × 10^{-3} cm and 30% of the animals showed CPS whilst at the same sensitizing dose an elicitation dose of 2.16 J/cm^2 produced ear swelling of 16.5 ± 8.4 × 10^{-3} cm and 100% of the animals developed CPS. CPS was defined as ear swelling >3 S.D. of that seen in non sensitized animals. Intermediate elicitation doses produced intermediate responses. Similar results were seen with increasing elicitation doses at sensitization doses above 180 mJ/cm^2.

In our study a 100% rate of CPS to TCSA was consistently achieved with sensitizing doses of UVA of 180 mJ/cm^2 or more and in order to detect the CPS the elicitation dose of UVA had to be 2.16 J/cm^2.

12. Tumor Antigen Expression in the Skin of Mice Exposed to Ultraviolet Radiation. K. H. SEILSTAD, C. W. DEWITT, G. G. KRUEGER, AND L. K. ROBERTS, Depts. of Internal Medicine (Dermatology) and Pathology, University of Utah School of Medicine, Salt Lake City, UT.

It has been postulated that prior to tumor emergence, antigens develop in the skin of mice exposed to subcarcinogenic doses of ultraviolet radiation (subUV). These antigens are capable of eliciting immunologic responses that are cross-reactive with UV-tumor associated transplantation antigens (TATA). In this study, a battery of monoclonal antibodies (MAb) generated against the TATA expressed by UV-tumors that were induced in C3H and related H-2 congenic strains of mice were used to identify TATA expression in the skin of C3H mice exposed to subUV. Analyses by indirect immunoperoxidase and indirect immunofluorescence staining did not provide visual detection of TATA in either epidermal sheets or frozen sections of skin from subUV C3H mice. However, cross-reactive TATA were detected in an ELISA assay employing either 3 M KCl solubilized cell extracts from the epidermis of C3H mice exposed to subUV or supernatants from UV-tumor cell cultures. These antigens were not present in solubilized epidermal cell extracts from non-UV-irradiated mice. Class I molecules (H-2D) were identified by this method in these samples. These antigens (TATA and Class I) were not detected in supernatant from epidermal cell tissue cultures (from subUV and normal C3H mice). From this study we conclude that: cross-reactive TATA are expressed in the epidermis of mice exposed to subUV; a limited number of cross-reactive TATA determinants are expressed by cells within subUV skin, since only 1 of 5 TATA reactive MAb recognized these antigens; and these antigens were detected in the supernatant from cultured UV-tumor, but not cultured epidermal cells.

13. Immune Suppression Mediated by Extracorporeal Photopheresis. C. L. BERGER AND R. L. EDELSON, Dept. of Dermatology, Columbia University, New York, NY.

Eight patients with leukemic cutaneous T cell lymphoma (CTCL) and one patient with the autoimmune disease pemphigus vulgarus have been reported to respond to extracorporeal photopheresis. This treatment exposes a lymphocyte-enriched blood fraction to 8-methoxypsoralen and ultraviolet A light, prior to returning cells to the patient.

Response to therapy was monitored by *in vitro* evaluation of cell viability, response to phytohemagglutinin (PHA), cytofluorographic detection of the binding of monoclonal antibody BE2 (tumor-associated marker) and alterations in T cells analyzed by monoclonal antibodies T3 (Pan), T4 (Inducer) and T8 (Suppressor/Cytotoxic).

The efficacy of phototherapy has been demonstrated in all patients by a gradual decrease in lymphocyte viability *in vitro* from 91% + 1 Pretx to 12% ± 1 on Day 3, and by 98% ± 3 inhibition of PHA response. An *in vivo* reduction in mature T cells was demonstrated by an acute decline in circulating T cells (46% reduction ± 29), $T4^+$ cells (55% ± 23) and $T8^+$ cells (40% ± 30) in 4 patients. Decreased tumor burden was demonstrated by >50% reduction in $BE2^+$ cells maintained in the bloodstream of each of 2 reactive patients for more than 2 years. A 35% ± 6 reduction in the absolute number of circulating lymphocytes also occurred in 4 CTCL patients treated for 2–3 years. Most indicative of the immunosuppressive nature of photopheresis is the three-fold increase in $T8^+$ cells seen in the circulation of 5 long-term patients.

These results suggest that extracorporeal phototherapy mediates long-term cytoreduction of leukemic tumor burden in intractable CTCL. This therapy is immunosuppressive and potentially useful in treatment of autoimmune disorders, as well as leukemias.

14. Effect of β-NAD Addition on the Post-Ultraviolet Light Hypersensitivity of Cockayne Syndrome Lymphoblastoid Cell Lines. FUKIO OTSUKA, ATSUSHI KUKITA, AND JAY H. ROBBINS, Dept. of Dermatology, Tokyo University Hospital, Tokyo, Japan, and Dermatology Branch, NIH, Bethesda, MD.

Cultured cells from Cockayne syndrome (CS) patients are known to have a high lethality to 254nm ultraviolet (UV) light. It is controversial if the exogenous β-NAD normalize such an abnormality of CS fibroblasts. We have reported CS lymphoblastoid cells to be reliable to evaluate the UV sensitivity in terms of survival assay. Thus we studied the post-UV survival of CS lymphoblastoid lines added with β-NAD.

4 CS, 2 xeroderma pigmentosum (XP) and 3 normal control lines, irradiated with 1–10 J/m^2 254 nm UV light or mock irradiated, were cultured with or without 0.1 mM β-NAD. A line's post-UV viability ratio was calculated by dividing the number of viable cells in an irradiated culture on the 3rd postirradiation day by the number of viable cells in an unirradiated culture of the same line on that day. Viability ratios and Do values were compared between cultures with and without β-NAD.

2 CS lines showed a significant increase of the post-UV survival by β-NAD, however it could not rectify the UV sensitivity of CS nearly to the normal level. The other 2 CS, 2 XP and 3 normal control lines did not respond to β-NAD addition.

It is concluded that CS lines are heterogenous in the response of β-NAD addition and β-NAD may play a partial role, in reducing the UV lethality to some extent in some CS lines.

15. Action Spectrum and Molecular Weight of Photoantigens in Solar Urticaria. TOSHIYUKI AOKI, Dept. of Dermatology, Habikino Hospital of Osaka Prefecture, Osaka, Japan.

Five cases of solar urticaria were investigated monochromatically and immunologically. Patient's serum was irradiated with projector light and injected into the skin of the patient own. Immediate wheal and flare reaction was noted in four of five cases. In the remaining one only irradiated plasma gave the positive reaction. These serum and plasma did not induce any reaction in normal subjects, and also irradiated serum or plasma of the normal subjects caused positive reaction only in the patients. Thus antigen was thought to be produced in the serum or plasma by light irradiation (photoantigen).

Molecular weight determination of photoantigens by Sephadex G-200 gel-filtration of irradiated serum or plasma and monochromatic action spectrum study showed that in four cases with photosensitivity at 400–500 nm, molecular weight of photoantigen was 25,000–45,000 dalton, and in one of four cases who showed additional photosensitivity at 330–380 nm another photoantigen was noted in the range 300,000–1,000,000 dalton. In the case in which photoangiten was produced only

in plasma, action spectrum ranged from 290 to over 600 nm and Sephadex G-200 gel-filtration could not localize antigen activity.

These results seem to implicate that action spectrum of solar urticaria is related to the nature of photoantigens and in particular case to the molecular weight of photoantigens.

CONCURRENT SESSION 4B: KERATINIZATION

Federal

Larry Peterson and Tadashi Tezuka, Presiding

1. Analysis of Keratin Compositions of Normal Human Epidermis and Callus by Two-Dimensional Gel Electrophoresis and Anti-Keratin Monoclonal Antibodies. S. DEKIO, G. J. STEVENS, K. HASHIMOTO, AND J. JIDOI, Dept. of Dermatology, Wayne State University, Detroit, MI, VAMC, Allen Park, MI, and Dept. of Dermatology, Shimane Medical University, Izumo, Japan.

Keratin compositions of normal human epidermis (NHE) and of callus (C) were analysed by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). NHE keratins were separated into 5 neutral and 24 acidic components, and C keratins were separated into 2 neutral and 17 acidic components. The 50 kd and 58 kd keratins both having neutral and acidic components on 2D-PAGE of NHE and/or C keratins were analysed by immunoblotting using anti-keratin monoclonal antibodies, EKH4 (50 kd) and AN3 (50 kd and 58 kd), which recognized keratins in the lower epidermal layers. Although EKH4 and AN3 stained 50 kd-pI 7.0 and 50 kd-pI 6.3 components of NHE, they did not stain 50 kd components of C. Since 50 kd keratin consisted of one neutral and two acidic components in NHE and one acidic component in C, one of the two acidic components of NHE and the acidic component of C were not recognized by these antibodies, suggesting their antigenic difference from the stained 50 kd components. Although AN3 stained 58 kd-pI 7.0 component of NHE and C, it stained also 56 kd-pI 6.2 component of C. The fact that acidic 58 kd component(s), a newly found one in the present study, were not stained by AN3 suggested that they had antigenic difference from the neutral 58 kd component. The 56 kd-pI 6.2 component of C might be a degraded neutral 58 kd component.

The present study has shown that 2D-PAGE combined with immunoblotting using anti-keratin monoclonal antibodies is an excellent method for the analysis of skin keratins.

2. On the Expression of Low Molecular Weight Keratins in Epidermis. E. BIRGITTE LANE, IRENE M. LEIGH, AND CAROL A. MAKIN, Imperial Cancer Research Fund, London, U.K.

The keratin proteins which they express divide epithelia between the simple epithelial of internal organs and the stratified squamous epithelium of epidermis, and it is widely held that the 3 smallest, embryologically primary keratins, nos. 8, 18 and 19 (= LMW keratins) hallmarks of simple epithelia, are not expressed in normal adult keratinocytes, although they are in many transformed derivatives. (Merkel cell non-keratinocytes do express these keratins.) With recent new data from high avidity- and monospecific-monoclonal antibodies to keratins and biotin-avidin detection methods, earlier reports on this phenomenon have been re-evaluated. Using LP34 (multispecific), LE61 (to 18), LE41 (to 8), LP2K (to 19) and CAM5.2 (to 8 and 18) as indicator antibodies, LMW keratins have been unequivocally detected in keratinocytes in normal differentiating fetal epidermis and adult hair follicle cells. Local variations can be seen which are masked in biochemical analysis. Persistent low-level expression of LMW keratins in adult keratinocytes suggests that loss of keratins during differentiation might only be a quantitative reduction.

3. Structure and Assembly of Human Epidermal Keratin Filaments. RIVA EICHNER, EMMANUEL C. GLAVARIS, AND UELI AEBI, Depts. of Dermatology and Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD.

To elucidate the structure and assembly of keratin filaments, keratins extracted from human epidermal cells were renatured *in vitro* and examined by electron microscopy. During "open dialysis" against 5 mM Tris, 1 mM DTT, pH 7.5 (standard conditions), very short 2.5–4.5 nm diameter protofilaments or protofibrils and 10 nm filament pieces were initially observed (20–45 min), followed by the appearance of full-length filaments (by 100 min). By increasing the pH of the dialysis buffer, or including 2–6 M urea, the process of filament assembly could be retarded or arrested. In all cases, predominantly unit-length (40–100 nm) structures accumulated as stable intermediates. These short structures rapidly formed long filaments when dialyzed against standard conditions. The diameter of full-length filaments ranged between 8–12 nm, even along individual filaments. When reassembled filaments were dialyzed against various buffers (e.g. 10 mM PO_4, pH 7.5 or 5 mM Tris, pH 9.5), the filaments unraveled and gradually dissociated into thin, short subunits, although not necessarily in the reverse sequence of assembly.

These data suggest that *in vitro* keratin filament assembly is a multistep process: first lateral aggregation of keratin polypeptides to form 2–3 nm protofilaments → 4.5 nm protofibrils → 10 nm filaments of unit length, followed by longitudinal annealing of filament pieces to form full-length filaments. The variation observed in filament width suggests that the number of protofilaments per 10 nm filament is not rigidly controlled. Our results further suggest that keratin filaments are dynamic structures which may be modulated and remodeled by their environment.

4. Reorganization of Keratin-Intermediate Filaments and Microtubules During Cell Cycle and Cytostatic Drug Treatments in Human Keratinocytes. YASUO KITAJIMA, SHUNICHIRO INOUE, AND HIDEO YAOITA, Dept. of Dermatology, Jichi Medical School, Tochigi, Japan.

The functions of keratin-intermediate filaments (KIFs) remain unclear compared to microtubules (MTs) and microfilaments (MFs). This may be due to lack of biochemical agents that disrupt the function of KIFs in manner analogous to that of colchicine and cytochalasin B. From functional aspects, we have studied the behavior of KIFs during cell cycle and disruption of MTs and MFs.

Normal human keratinocytes were cultured on glass coverslips. Immunofluorescence microscopy by double-staining with anti-keratin and antitubulin showed different networks between KIFs and MTs in cells in colony, but similar arrangements in migrating single cells. They underwent the structural rearrangement independently during mitosis. In prophase, two poles (MTs) for spindle were formed first without KIF rearrangements. In rounded, meta- and anaphase cells, KIFs formed a grobal aggregation (a cage) around the spindle and a spoke-like array radiating from the cage to the cell periphery where they were connected with those of the adjacent cells at desmosomal sites. In telophase, KIFs and MTs reverted back independently to the original organization. These results may suggest that KIFs play a role in a cell societal function to keep the cell in its correct position during mitosis, while MTs engage in intracellular division. By independent 1-hr treatment with colchicine (20 μM) or cytochalasin B (20 μM), almost no effects were exerted on KIF arrays, while MTs or MFs were disrupted. The simultaneous treatments induced an interesting reorganization of KIFs into a basket-weave like structure. These results may suggest that KIFs are dinamic rather than static structures in association with other cytoskeletons.

5. Intermediates in the Conversion of Prekeratin into Keratin Molecules in Normal Human Epidermis and Psoriasis. YOHTARO KATAGATA AND KAZUO ASO, Dept. of Dermatology, Yamagata University School of Medicine, Yamagata, Japan.

In order to elucidate the relationship between prekeratin (PK) and keratin (KF) molecules, we performed pulse-chase experiments using ^{35}S-methionine or ^{14}C-glycine in human epidermis in vitro. Of six PK molecules (49, 52, 55, 62, 69 & 71K) that incorporated ^{35}S-Met, 55K-PK incorporated the most ^{35}S-Met. In three molecules (52, 55 & 62K), incorporation was decreased at 30 min after chasing; however, incorporation of only two (55 & 62K) of the six PK molecules was increased at 60 min. On the other hand, in the case of ^{14}C-Gly, the two PK molecules (52 & 62K) were increased instantly without decrease after chasing, and reached a peak at 60 min. From these results, we concluded that the initial stage of processing is as follows: These PK molecules (52, 55 & 62 K) are first cleaved in the N-terminal region, then two PK molecules (55 & 69K) proceed to intermediates (52 & 62K) by means of some proteolytic enzyme(s).

Moreover, a similar experiment was undertaken in psoriasis vulgaris using ^{14}C-Gly. In addition to the above six PK molecules, another molecule (46K) was detected in fluorography. Incorporation of ^{14}C-Gly was almost the same as that in normal epidermis, but the 62 and 71K molecules were increased at 120 min after chasing. The increase in 71K and stability of 52K after chasing may indicate an abnormality or deficiency in an enzyme that converts PK into KF molecules in normal epidermis.

6. Induction of Psoriasis-like Keratin Expression in Normal Epidermis by Tape-Stripping. ROBERT A. WEISS, ABEL TORRES, AND RIVA EICHNER, Depts. of Dermatology, Johns Hopkins University School of Medicine, Baltimore, MD, and NYU School of Medicine, New York, NY.

To better understand the biologic significance of keratins expressed in epidermal hyperproliferative disorders, we analyzed keratins from normal epidermis which was induced to hyperproliferate by tape-stripping the stratum corneum. Stratum corneum was removed by repeated cellophane tape-stripping from the backs of three normal volunteers and biopsy specimens of these areas were taken at 0, 1, 2, 3 and 7 days. Keratins were extracted, separated by SDS-gel electrophoresis and further characterized by immunoblot analysis using monoclonal antikeratin antibodies, AE1 and AE3. In addition, corresponding cryostat sections from each biopsy were stained by indirect immunofluorescence (IF) with the same monoclonal antibodies. Our results indicated that whereas normal epidermis expressed four major keratins (50 kd, 56.5 kd, 58 kd and 65–67 kd), by the first day hyperproliferative tape-stripped epidermis expressed the additional keratins, 48 kd (AE1-positive) and 56 kd (AE3-positive). These same keratins were previously identified in psoriasis and other hyperproliferative epidermal disorders. IF staining revealed a shift from the normal AE1 basal layer pattern to intense AE1 suprabasal staining as previously reported for hyperproliferative epidermal diseases. Both the patterns of keratin expression and IF staining returned to virtually normal by day 7. These results indicate that expression of the 48 kd and 56 kd keratins and associated AE1 suprabasal staining, previously reported to be a consequence of epidermal hyperproliferative disease, may be induced in a reversible way in normal skin. This provides additional evidence that these keratins represent molecular markers for hyperproliferation.

7. A New Keratinocyte Protein Induced by Recombinant Human Gamma Interferon. J. N. MANSBRIDGE, B. NICKOLOFF, AND V. B. MORHENN, Psoriasis Research Institute and Dept. of Dermatology, Stanford University School of Medicine, Stanford, CA.

We have shown previously that recombinant human γ-interferon (r-INF-γ) causes the synthesis and expression of HLA-DR in cultured human keratinocytes within 3 days. In a search for further proteins induced by r-INF-γ, we have compared the electrophoretic patterns of proteins synthesized by keratinocytes labelled with ^{35}S-methionine in the presence or absence of r-INF-γ.

One-dimensional gels of triton-soluble proteins revealed a major new component of about 53,000 daltons induced within 24–48 h by r-INF-γ. Under conditions of saturating r-INF-γ concentration, this protein represented as much as 8% of the total triton-soluble ^{35}S-methionine incorporation. The induction was sensitive to α-amanitin and actinomycin D and so required RNA synthesis. The concentration of r-IFN-γ giving half-maximal induction of the 53,000 dalton protein was the same as that required for half-maximal surface expression of HLA-DR in the same experiment. The results suggest that the two systems depend on a common activation system.

Two-dimensional gels showed a number of r-IFN-γ-induced changes in addition to a major new component corresponding to the 53,000 dalton protein seen on one-dimensional gels. From comparison of gel patterns it is unlikely that the protein results from an altered post-translational modification. It is induced to a lesser extent by α- and β-interferon and a prominent protein with a similar molecular weight is induced in fibroblasts by γ-interferon.

8. Keratin Differentiation in Anagen Hair and Hair Follicle Studied with Anti-Hair Keratin Monoclonal Antibodies, HKN-2, 4, 5, and 7. KAORU ITO, MASAAKI ITO, TOSHIO TAZAWA, NAOYA SHIMIZU, KAORU KATSUUMI, AND YOSHIO SATO, Dept. of Dermatology, Niigata University School of Medicine, Niigata, Japan.

The purpose of this study was to investigate the immunological characteristics of hair fibrous proteins (HFPs) in anagen hair and hair follicle using anti-hair keratin monoclonal antibodies. The distribution of keratin filaments was ultrastructurally examined. HFPs were biochemically extracted from normal human hairs. By a mouse-hybridoma technique, four monoclonal antibodies, HKN-2, 4, 5 and 7, against HFPs were produced. Epidermal fibrous proteins (EFPs) were also extracted from human sole callus horny materials. By immunoblot analyses, HKN-2 and 4 decorated the electrophoretic bands of either HFPs or EFPs; however, HKN-5 and 7 reacted only with the bands of HFPs. Immunohistochemically, the frozen sections of human skin, intestine, kidney and liver tissues were examined. All four monoclonal antibodies stained the medulla, cortex, cuticle and inner root sheath in the keratogenous zone of anagen hairs. The reaction of KHN-7 was limited to these tissues, while KHN-5 reacted with one more layer, the innermost cell layer of the outer root sheath. Although HKN-2 showed reactions with various skin epithelial tissues except for epidermal basal cells and secretory cells of sweat glands, the reactivity of HKN-2 was limited within the skin. HKN-4 displayed a broad cross-reactivity with all of the skin epithelial cells and other various simple epithelial cells. Ultrastructurally, the innermost cells showed a different distribution pattern of keratin filaments from that of the other outer root sheath cells. These findings indicate that some components of HFPs are immunologically specific for hair cells, while others are common to other epithelial cells. The innermost cell layer of the outer root sheath may be an unique layer in cell differentiation.

9. The Specificity of Naturally Occurring Antikeratin Antibodies in Human Sera: Comparative Studies with Different Methods. KEIJI IWATSUKI*, JACQUELINE VIAC, ALAIN RÉANO, MARIE-JEANNE STAQUET, AND JEAN THIVOLET, *Dept. of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan, and INSERM U 209, Hôpital E. Herriot, Lyon, France.

The specificity of the so-called antiepidermal antibodies (Ab) found in human sera was studied using 3 methods : immunoblotting, enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM). After a screening test by indirect immunofluorescence (IF) on more than 70 sera obtained from patients with various diseases and controls, sera may be classified in five different groups according to the IF pattern on epidermis ; sera reactive with (1) stratum corneum (SC), (2) upper layers (U-Cyt), (3) whole epidermis (G-Cyt), (4) basal cells (B-Cyt) and (5) negative ones. By immunoblotting, all the 22 IF-positive sera were found to bind to one or more keratin bands, and did not show any reactivity with epidermal soluble proteins. SC-Ab were mainly directed against a 67 Kd keratin band, while U-Cyt and G-Cyt-Ab bound to both 58-56 Kd and 67-63 Kd keratins. B-Cyt-Ab reacted strongly with 63 Kd keratins and slightly with a 50 Kd band. Moreover, antikeratin Ab have been detected by immunoblotting in the IF-negative sera. The ELISA study showed that most of the IF-positive sera contain high titers of antikeratin Ab. The IEM study using sera containing U-Cyt or B-Cyt-Ab demonstrated two distinct reaction patterns ; U-Cyt-Ab stained tonofilaments of suprabasal keratinocytes, while B-Cyt-Ab characteristically reacted with those of basal cells. In conclusion, (a) human antiepidermal Ab are directed against different keratin polypeptides, (b) immunoblot is the most sensitive technique, (c) antikeratin Ab commonly occur in almost all human sera.

10. Measurement of In Vivo Mouse Epidermal Protein Synthesis. CHARLES S. HARMON, JUNG H. PARK, AND CYNTHIA MARCELO, Dept. of Dermatology, University of Michigan Medical School, Ann Arbor, MI.

A method for measuring the absolute rate of *in vivo* epidermal protein synthesis has been developed. To determine the absolute synthesis rate the specific activity (SA) of the precursor pool of labelled amino acid must be known throughout the labelling period. A large dose of ^{3}H-phenylalanine (Phe, 150 umoles/100 gm) was injected i.v. and after 1, 2, 5, 10, 20 and 30 min epidermal samples were obtained with a keratome, frozen, and homogenized in 2% PCA. The Phe SA was determined in: 1) plasma, 2) the epidermal free pool, and 3) the protein fraction after 6 M HCl hydrolysis. Phe was assayed by decarboxylation to phenylethylamine and fluorimetric analysis. After injection, the SA of plasma Phe decreased linearly while that of the free pool reached a plateau within 2 min, remained constant for 10 min, and then declined. Flooding of the epidermal free Phe pool was shown by the rapid rise of the free/plasma SA ratio, to a maximum of 0.79 at 10 min. The results showed that epidermal protein synthesis was linear over 30 min, with a fractional rate of 81% per day and a rate of 86.8 μg protein/mg wet weight/day. The RNA-based synthesis rate, a measure of ribosomal efficiency, was 21.0 μg protein/mg RNA. The large dose of Phe used did not itself stimulate protein synthesis, since injection of an equal dose of unlabelled Phe did not increase incorporation of tracer doses of ^{3}H-lysine and ^{14}C-threonine. The results show that epidermis is highly active in protein synthesis and the method described here will be used to study the regulation of epidermal protein synthesis at the level of translation.

11. The Processing of Filaggrins by Cultured Human and Rat Keratinocytes. HOWARD P. BADEN, JOSEPH KUBILUS, AND IAN SCOTT*, Dept. of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, and *Unilever Research, Bedford, U.K.

Profilaggrin (PF) is synthesized as a high molecular weight (>300,000) polypeptide in the granular layer of epidermis and is de-

graded to filaggrin (F) which induces aggregation of keratin filaments. We have looked for PF and F in cultured keratinocytes since these cells represent a potentially valuable model for studying PF metabolism. A monospecific antibody was prepared to purified rat F (AbRF) and a monoclonal antibody, HF-1, to human F. Newborn rat and human keratinocytes were grown in supplemented Dulbecco's MEM. Extracts of cultured cells and epidermis were prepared with 2% SDS buffer containing PMSF. By indirect immunofluorescence AbRF reacted with the granular layer of rat but not human epidermis and the reverse was true for HF-1. Immunoblots of extract of both newborn rat epidermis and cultured keratinocytes showed a reaction with F, PF and a number of their breakdown products. Immunoblots of human epidermal extracts showed a reaction to a polypeptide of Mr (>300,000), F and several polypeptides of intermediate molecular weight, while those of cultured keratinocytes showed a reaction primarily to the high molecular weight polypeptide and the intermediates. Absorption of HF-1 with purified human F blocked all its immunoblot reactions demonstrating that all reacting species were immunologically related to F.

Our ability to measure PF and its degradation products in cultured keratinocytes affords the opportunity to determine the effect of natural and synthetic compounds on PF and F formation and degradation in epidermal cells in a controlled environment.

12. Changes in Histidine-Rich Protein (HRP) Associated with Formation of Keratohyalin Granules (KG) and Cornified Cells. I. P. S. DHILLON, M. NAKAGAWA, K. FUKUYAMA, AND W. L. EPSTEIN, Dept. of Dermatology, University of California, San Francisco, CA.

HRP is a basic protein that displays various M_r and solubility characteristics in different epidermal cell layers. In KG HRP is less soluble with higher M_r (>54K) than in cornified cells where it is more soluble and has a lower M_r (<50K). In this study we compared HRP in rat skin after birth to HRP in two stages of fetal development—Stage 1: before KG appeared (7–11 days gestation) and Stage 2: before cornified cells formed (15–17 days gestation). Skin proteins were extracted stepwise in 1) 20 mM PIPES, pH 6.8, containing 0.14 M NaCl and 1 mM each of diisopropylfluorophosphate and p-chloromercuribenzoate (soluble HRP) and 2) 0.1 M Tris-HCl, pH 8.0, containing 2 M KSCN and the inhibitors (bound HRP). Extracted proteins were separated by SDS-PAGE, blotted on nitrocellulose membrane, and HRP identified with rabbit anticornified cell HRP serum. Predominant HRP M_rs were 300–70K, 70–55K and <50K. Their appearance differed according to level of development.

	Soluble HRP			Bound HRP		
	300–70K	70–55K	<50K	300–70K	70–55K	<50K
Stage 1:	—	+++	−	−~+	+++	−
Stage 2:	−	+++	−	+++	++	+
After birth:	−	+	+++	+++	++	++

This study shows that the M_r of HRP in the skin before KG formation was primarily 70–55K. The appearance of KG was associated with an increase of high and low M_r HRP in the bound fraction while cornification resulted in increased solubility of HRP with low M_r.

13. The Presence of a Spectrin-like Protein in Murine Epidermis. AMELIA H. KAYMEN, JEANNETTE M. BONIFAS, KIMIE FUKUYAMA, AND ERVIN H. EPSTEIN, JR., Dept. of Dermatology, San Francisco General Hospital and University of California Medical School, San Francisco, CA.

Spectrin is the major protein of the RBC membrane skeleton. Abnormalities of this protein render the membrane skeleton unstable, causing fragility and hemolysis. Proteins similar in structure and antigenically related to spectrin have been found in some non-RBC tissues. We have studied whether epidermis also contains such spectrin-like proteins.

Spectrin, along with several other membrane skeletal proteins, was extracted from mouse RBC ghosts with a low ionic strength buffer consisting of 0.1 mM sodium phosphate, 0.1 mM EDTA, 0.2 mM PMSF, pH 8.0. This extract was injected into rabbits to elicit antibodies.

Newborn mouse epidermis, separated from dermis after treatment with dithiothreitol, was homogenized, washed and then extracted under the same conditions as used for the RBCs. On SDS-PAGE the epidermal extract contains many polypeptides, among which is a protein that migrates as a doublet of MW 240,220 kd. and closely resembles RBC spectrin. After transfer to nitrocellulose membrane this doublet stains intensely with the rabbit antibody to the RBC extract. This demonstrates that the epidermal spectrin is immunologically cross reactive with RBC proteins. Immunoperoxidase staining detects the rabbit antibody bound to cells of the upper spinous, granular, and horny layers of frozen sections of mouse skin, with accentuation at the cell peripheries.

We conclude that the epidermis contains a protein related to RBC spectrin. Its cellular location suggests that this protein may contribute to the structural integrity of keratinocytes, much as spectrin contributes to the structural integrity of RBCs.

14. A Novel Cell Membrane Coating Protein of Newborn Rat Stratum Corneum Cells. MASAE TAKAHASHI AND TADASHI TEZUKA, Dept. of Dermatology, Kinki University School of Medicine, Osaka, Japan.

Hematoxylin stain renders keratohyalin granules positive outside of the stratum corneum but negative when inside the stratum corneum. The aim of this experiment was the extraction of a hematoxylin positive substance from keratohyalin granules and the identification of its location in the stratum corneum. The epidermis of a 3-day old rat was separated by NH_4Cl solution, frozen in liquid nitrogen, lyophilized and powdered by a Willy mill. Powdered epidermis was then extracted in a 50 mM Tris-HCl buffer at pH 7.3 overnight at room temperature. Centrifugation followed at 15,000 g for 10 min. After nucleic acids were eliminated by Poly (U)-Sepharose 4B column chromatography, the supernatant fraction was purified by preparative isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electro phoresis (SDS-PAGE). The protein band was physically separated and SDS was removed via Henderson's technique. The molecular weight of hematoxylin protein was 14,500 daltons and had an isoelectric point of 4.7. The quantities of resultant serine, glutamic acid, glycine, histidine and halfcystine residues were 11.0%, 13.0%, 15.0%, 1.9% and 0.7% respectively. The protein located itself on the cell membrane region of the whole stratum corneum, as per immunofluorescence examination. This hematoxylin protein could be the precursor of the stratum corneum membrane proteins.

15. A Previously Undescribed Antigenic Component(s) Is Present in Human Cornified Envelope. JOSEPH KUBILUS, JOSEPH KVEDAR, AND HOWARD P. BADEN, Dept. of Dermatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

To probe the structure of the cornified envelope and to identify its soluble precursors, polyclonal antibodies were prepared in rabbits (ACEr) and mice (ACEm) to the cornified envelope of cultured human keratinocytes. A polyclonal antibody to involucrin (AI), a protein reported to be a human envelope precursor, was also prepared in rabbits. By indirect immunofluorescence (IIF), AI and ACE's showed a peripheral staining pattern with human epidermis while the ACE's also reacted with rat and cow epidermis. AI and the ACE's stained the upper epidermal layers but the ACE's also showed weak reactivity to the lower layers. This difference was more clearly seen in a variety of benign and malignant epidermal tumors. Both the ACE's and AI reacted strongly with the involucrin of neutral extracts of cultured human keratinocytes by immunoblotting but no reaction was seen with similar extracts of newborn rat epidermis or cultured keratinocytes. When ACEr was absorbed with purified involucrin, its reaction to this protein was lost by dot blot and ELISA. However, its reactivity to human, cow and rat epidermis was unchanged when examined by IIF. Using the immunized mice, we have prepared two monoclonal antibodies which produce the peripheral staining pattern with human epidermis. Both of these antibodies are unreactive to involucrin by ELISA.

These results establish that involucrin is contained in human cornified envelopes and demonstrate a previously undescribed envelope antigen(s) common to several species. With the demonstration that monoclonal antibodies can be developed to cornified envelopes, we have a new approach for detecting additional envelope precursors.

CONCURRENT SESSION 4C: INFLAMMATORY MEDIATORS

South American

Takashi Aoyagi and Kim Yancy, Presiding

1. Increased C3a and C4a Anaphylatoxins in Psoriatic Scales and Sera. HIDEAKI TAKEMATSU, KYOKO OHKOHCHI, AND HACHIRO TAGAMI, Dept. of Dermatology, Tohoku University School of Medicine, Sendai, Japan.

Although we have shown that the stratum corneum from the psoriatic lesions contains chemotactic anaphylatoxin (C5a) which is pre-

sumed to be responsible for characteristic transepidermal migration of leukocytes towards the horny layer, controversy continues as to the mechanisms underlying the generation of C5a in psoriatic lesions. The purpose of this report is to determine which complement pathway plays a major role in the production of C5a using radioimmunoassay for anaphylatoxin. Direct determination of the amount of cleavage products, C3a and C4a, can assess the extent of involvement of each pathway.

Using radioimmunoassay technique, we measured the levels of C3a and C4a anaphylatoxins in the scale extracts from psoriasis and pustular psoriasis as well as in the sera of 46 psoriatic patients and of 26 healthy volunteers. The C3a and C4a contents in these scale extracts were significantly higher than that of normal horny layer (e.g. average C3a and C4a levels in psoriatic scales vs control were 547 ± 121 vs 20 ± 8 and 457 ± 72 vs 13 ± 6 ng/ml, respectively). The serum concentrations of C3a and C4a were also significantly higher than that of non-psoriatic controls (C3a 259 ± 29 vs 146 ± 13; C4a 351 ± 42 vs 199 ± 30 ng/ml). Although a correlation between the serum levels of anaphylatoxins and severity of skin lesions was noted in each patient, it became unclear when they were compared as a whole population.

These data suggest complement activation via the classical pathway takes place in the lesional skin as well as in the body and that there is a possibility that the skin lesions are not the sole activation site of complement.

2. Two Forms of Keratin Hydrolases in Psoriatic Epidermis: Interaction with Epidermal Trypsin Inhibitor. T. HIBINO, S. IZAKI, AND M. IZAKI, Dept. of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan.

Two forms of keratin hydrolases (KH-I and KH-II) were demonstrated in psoriatic scale extract. In addition, effect of epidermal trypsin inhibitor on KH-I and KH-II were investigated. Keratin hydrolyzing activity was assayed with keratin-agarose plate method and Ile-Pro-Arg-p-nitroanilide. Psoriatic scale extract was fractionated by 50% ammonium sulfate precipitation, passing through lysine-Sepharose, DEAE Sepharose, Sephacryl S-200, and aprotinin-Sepharose affinity chromatography. At the final purification step, keratin hydrolyzing activity was found in adsorbed (KH-I) and non-adsorbed fraction (KH-II). The ratio between KH-I and KH-II was found approximately 4 to 6. KH-I showed a single protein band at molecular weight of 30,000 with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Furthermore, in an electrophoretic enzymography method with keratin-polyacrylamide slab gels, KH-I demonstrated a translucent band at molecular weight of 30,000 indicating keratin digestion. The purified KH-I showed high level of hydrolytic activity on Ile-Pro-Arg-p-nitroanilide and other peptidyl arginine substrates and was found to be a serine proteinase inactivated by DFP, antipain, soybean trypsin inhibitor and aprotinin. On the other hand, KH-II, a nonadsorbed fraction, showed multiple protein bands with SDS-PAGE, while broad and diffuse translucent bands in higher molecular weights were shown with the enzymography method. Substrate specificity and inhibitor susceptibility for KH-II were found very similar to those of KH-I. However, epidermal trypsin inhibitor distinguished KH-I from KH-II: no effect on purified KH-I demonstrated, whereas complete inhibition for KH-II was revealed.

3. Uninvolved Psoriatic Epidermis Has Increased Capacity to Synthesize 12-Hydroxy-eicosatetraenoic Acid. KNUD KRAGBALLE, LARRY DESJARLAIS, DAVID E. ALTMAN, AND JOHN J. VOORHEES, Dept. of Dermatology, University of Michigan Medical School, Ann Arbor, MI.

Certain arachidonic acid metabolites (leukotriene B_4 (LTB_4) and 12-hydroxyeicosatetraenoic acid (12-HETE)) detected in psoriatic lesions have the capacity to induce skin inflammation characterized by neutrophil exocytosis. The purpose of this study was to determine the capacity of uninvolved psoriatic epidermis to generate these lipoxygenase products in vitro. Keratome biopsies were obtained from untreated patients with psoriasis of less than 10% skin involvement. Biopsy site was at least 30 cm away from lesional skin. Epidermis was peeled off dermis after immersing the biopsy in PBS at 55°C for 25 sec. Homogenized epidermis was suspended in PBS with 2 mM glucose and 5 mM reduced glutathione at pH 7.4, and incubated with arachidonic acid [^{14}C]arachidonic acid and ionophore A23187 at 37°C for 10–30 min. Maximal transformation of arachidonic acid occurred at 25 μM arachidonic acid and 1 μM A23187. Extracted lipids were subjected to reversed-phase high-performance liquid chromatography (RP-HPLC) using a solvent phase of aqueous acetonitrile (30% to 85%). Chromatographic peaks were identified by their coelution with authentic standards and by UV spectrometry. In addition, compounds purified by RP-HPLC were assayed for immunoreactivity in RIA. 12-HETE was the major HETE generated by epidermis. Small amounts of 5-HETE and 15-HETE were detected in a few samples. The only LT-like compound formed by RP-HPLC eluted close to LTB_4, but had a retention time different from that of known LTs. This LT-like compound exhibited a triplet UV absorbtion spectrum with maximum at 274 nm. Compared with normal epidermis (mean 4.8 ng/mg wet tissue/30 min; n = 10), the 12-HETE formation by uninvolved psoriatic epidermis (mean 6.2 ng/mg wet tissue/30 min; n = 10) was significantly increased ($p < 0.02$). These results indicate that the 12-lipoxygenase pathway is highly active in human epidermis, and are compatible with the presence of an intrinsic abnormality of epidermal arachidonic acid metabolism in psoriasis.

4. Enzyme Preparations from Human Epidermis Lack the Capacity to Transform Linoleic Acid (18:2,n6) and Gammahomolinolenic Acid (20:3,n6) into Arachidonic Acid (20:4,n6). R. CHAPKIN, V. A. ZIBOH, C. L. MARCELO, AND J. J. VOORHEES, Depts. of Dermatology, University of California, Davis, CA, and University of Michigan, Ann Arbor, MI.

Although arachidonic acid (AA) is an essential constituent of human epidermal phospholipids and the precursor of the variety of eicosanoids in this tissue, its source in this tissue has remained unclear. We therefore tested whether or not linoleic acid (LA), a dietary precursor of AA, can undergo desaturation and elongation into AA by microsomal preparations prepared from keratome slices from normal human subjects. Epidermal microsomal preparations were incubated *in vitro* with ^{14}C-linoleic acid (18:2, n6) and ^{14}C-gammahomolinolenic acid (20:3, n6) acids respectively in parallel with rat liver microsomal preparations where activities of the Δ^6 and Δ^5 desaturases are well documented. The conversions of the ^{14}C-fatty acids were determined after methylation and separation of the ^{14}C-fatty acid methyl esters by argentation thin layer chromatography (TLC) and reverse phase high performance liquid chromatography (HPLC). Our data demonstrated that human epidermal microsomal preparations at similar protein concentrations as rat liver preparations lacked the capacity (<1%) to transform (i) ^{14}C-18:2n6 into ^{14}C-18:3n6. The enzyme which catalyzes this reaction is the ratelimiting Δ^6 desaturase; (ii) conversion of ^{14}C-20:3n6 into ^{14}C-20:4n6. The enzyme which catalyzes this reaction is the Δ^5 desaturase. These data are consistent with a recent report of the lack of Δ^6 and Δ^5 desaturases by microsomal preparations from rat and guinea pig epidermis. These findings indicate that epidermal arachidonic acid is not biosynthesized locally from tissue linoleic acid and must, therefore, depend on contribution from another endogenous source.

5. A Novel Eicosanoid Is the Major Arachidonic Acid Metabolite of Cultured Human Monocytes. MARC E. GOLDYNE, GENE F. BURRISH, AND CATHLEEN OLIVER, Depts. of Dermatology and Medicine, University of California, San Francisco, CA.

Monocyte-macrophages (MⒿ) can be a source of vasoactive and chemotactic eicosanoids in inflammatory skin disease. Our recent studies of arachidonic acid (AA) metabolism in MⒿ have led to the isolation of a novel eicosanoid. MⒿ (>95% pure) isolated from venous blood using a combination of Ficoll-Hypaque gradients and plastic adherence, were incubated in RPMI 1640 (10% fetal calf serum) for varying periods and then exposed to either ^{14}C-AA or ^{3}H-AA for 24 h. Lipid extracts of the culture supernatants were then subjected to thin-layer chromatography (TLC) in either of 2 solvent systems. A major radioactive peak (51% of total labeled product), seen only when MⒿ were present, comigrated with either prostaglandin D_2 or thromboxane B_2 depending on the solvent system used. However, unlike these known eicosanoids, this major peak was still generated in the presence of indomethacin (10^{-6} M). On reverse phase high pressure liquid chromatography, the unknown peak differed from the known leukotrienes, hydroxy acids, and their metabolites in having a unique retention time, a single UV absorption peak at 270 nm, and was not generated during a short (15 min) incubation with calcium ionophore A23187. MⒿ precultured for 3 weeks generated more unknown (80% of total labeled product) than did MⒿ precultured for 2 days. Thus, a major AA metabolite of human MⒿ is a heretofore uncharacterized eicosanoid that appears to be generated in greater quantity as the cells differentiate in culture. It has probably escaped prior recognition due to its co-migration with known eicosanoids when using routine TLC. Details of the structure and possible function(s) of this novel eicosanoid are currently under investigation.

6. Leukotriene B_5 Derived from Eicosapentaenoic Acid Does Not Stimulate DNA Synthesis of Cultured Human Keratinocytes, but Inhibits the Stimulation Induced by Leukotriene B_4. KNUD KRAGBALLE, JOHN J. VOORHEES, AND EDWARD J. GOETZL, Dept. of Dermatology, University of Michigan Medical School, Ann Arbor, MI, and Howard Hughes Medical Institute Laboratories, Dept. of Medicine, University of California Medical Center, San Francisco, CA.

Arachidonic acid derived leukotriene B_4 (LTB_4) is a potent stimulator of neutrophil chemotaxis and DNA synthesis of cultured human keratinocytes. Replacing eicosapentaenoic acid (active ingredient of fish oil) for arachidonic acid results in the formation of LTB_5 that is much less potent in stimulating neutrophils. We have studied the effect of LTB_5 on DNA synthesis of cultured human keratinocytes. LTB_5 was generated by guinea pig peritoneal PMN leukocytes stimulated with 100 μM eicosapentaenoic acid and 20 μM calcium ionophore A23187. Purified LTB_5 was added in ethanolic dilutions to confluent primary cultures of normal human keratinocytes. After incubation at 37°C for 72 hours DNA synthesis was determined by incorporation of [^{3}H] thymidine into DNA and by autoradiographic labeling indices. In a concentration range from 10^{-12} M to 10^{-7} M LTB_4 stimulated DNA synthesis. At similar concentrations LTB_5 failed to stimulate DNA synthesis. When an optimal concentration of LTB_4 (10^{-10} M) was incubated with LTB_5, a dose-dependent inhibition of the LTB_4-induced stimulation occurred. The mean inhibition was 21% at a ratio of LTB_5 to LTB_4 of 10:1, 33% at 100:1 and 54% at 1000:1. These results indicate that although LTB_5 by itself does not stimulate human keratinocyte DNA synthesis, it can interfere with the stimulation induced by LTB_4. Replacing eicosapentaenoic acid for arachidonic acid may, therefore, result in not only less inflammation, but also in less epidermopoiesis.

7. Leukotriene B_4 and 12-HETE: Induction of Pharmacological Tolerance and Clinical Significance. P. M. DOWD, A. KOBZA BLACK, P. M. WOOLLARD, AND M. W. GREAVES, Institute of Dermatology, London, U.K.

The pro-inflammatory and neutrophilotactic arachidonate lipoxygenase products LTB_4 and 12-HETE evoke intraepidermal microabscesses after topical application to healthy skin and are also present in increased concentrations in lesional skin of psoriasis. Clinical and histological responses to LTB_4 were similar in uninvolved skin of psoriatics (UP) and healthy control skin (HC) and multiple applications induced local tolerance. We have now examined the clinical and histological responses of UP and HC to single and repeated applications of 12-HETE alone and in combination with LTB_4. LTB_4 and 12 R, S, -HETE were applied to a 0.25 cm^2 area of intact forearm skin. 12-HETE dose response curves for area of erythema revealed no significant difference between UP and HC ($p > 0.5$). The ratio of the quantities of LTB_4 and 12-HETE required to evoke reproducible erythema and epidermal neutrophil infiltration of HC (50 ng and 20 μg respectively) approximated to the ratio of their concentrations in involved skin of psoriatics. Epidermal neutrophil infiltrations measured as no. of neutrophils per 10 h.p.f. produced by 20 μg 12 HETE were not significantly different in HC and UP (34.0 ± 18.31, 136.5 ± 75.4 mean ± s.e.m., n = 6 in both cases, $p > 0.5$).

The combination of 12-HETE and LTB_4 induced greater erythemal reactions than either agent alone, suggesting an additive effect but examination of neutrophil invasion of the epidermis revealed no significant additive effect over that produced by LTB_4 alone; reactions to the combinations in UP and HC did not differ significantly. Clinical and histological (See Table) tolerance was induced by repeated applications of 12-HETE and a combination of LTB_4 and 12-HETE.

Mean epidermal neutrophil counts/10 h.p.f. ± s.e.m. in response to

	(a) 20 μg 12-HETE		(b) 20 μg 12-HETE + 50 ng LTB_4	
	HC	UP	HC	UP
single	34.0 ± 18.3	136.5 ± 75.4	99.7 ± 30.2	320.3 ± 84.8
multiple	3.4 ± 2.1	1.9 ± 1.6	28.6 ± 18.1	25.3 ± 20.2

We conclude that a) clinical and histological responses of UP and HC to 12-HETE alone and in combination with LTB_4 are indistinguishable b) the concentrations of LTB_4 and 12-HETE found in lesional skin of psoriatics evoke equal intensities of epidermal neutrophil infiltration c) These results suggest that 12-HETE and LTB_4 are both important in inflammatory processes in psoriasis but are not solely responsible for induction of lesions.

8. Dermis Derived 15-Hydroxy-eicosatetraenoic Acid (15-HETE) Inhibits the 12-Lipoxygenase Activity of Human Epidermis. KNUD KRAGBALLE, LARRY DESJARLAIS, ELIZABETH A. DUELL, AND JOHN J. VOORHEES, Dept. of Dermatology, University of Michigan Medical School, Ann Arbor, MI.

By virtue of its capacity to inhibit the activity of 5- and 12-lipoxygenase activity of certain cell types, 15-hydroxyeicosatetraenoic acid (15-HETE) has been suggested a role in the regulation of arachidonic acid metabolism. We have studied the capacity of human skin to produce 15-HETE. Keratome biopsies were homogenized, suspended in PBS with 2 mM glucose and 5 mM reduced glutathione, and incubated with 25 μM arachidonic acid, [^{14}C]arachidonic acid and 1 μM calcium ionophore A23187. Incubations were carried out at 37°C for 30 min. Extracted lipids were subjected to reversed-phase high-performance liquid chromatography using acetonitrile (30–85%) in aqueous H_3PO_4 as the mobile phase. Chromatograms (as functions of UV absorbtion and [^{14}C]-radioactivity) produced a peak (maximal absorbtion at 238 nm) that coeluted with authentic 15-HETE. When this peak was collected and analyzed by RIA, it reacted as 15-HETE. To determine the source of 15-HETE, epidermis and dermis was separated (55°C for 25 sec) and analyzed separately. 88% to 100% of the 15-HETE was formed by the dermis. No other HETE was detected in dermis. Separation of epidermis and dermis by incubation with a protease from Streptomyces produced similar results, and primary cultures of human dermal fibroblasts incubated with arachidonic acid and A23187 synthesized predominantly 15-HETE. To assess whether 15-HETE can modify the arachidonic acid metabolism of human epidermis, synthetic 15-HETE was added to homogenized epidermis before incubating with 25 μM arachidonic acid and 1 μM A23187 for 30 min. 15-HETE inhibited 12-HETE formation in a dose-dependent manner ($ID_{50} = 3 \times 10^{-6}$M). Formation of prostaglandin E_2 ws not changed by 15-HETE. The ability of 15-HETE to modify the formation of chemotactic 12-HETE may be an important regulatory mechanism in the interaction between epidermis and dermis.

9. Formation of Immune Complexes at the Dermal-Epidermal Junction Is Dependent on Antigen Size and Charge. STEVE A. JOSELOW AND MART MANNIK, Division of Rheumatology, University of Washington, School of Medicine, Seattle, WA.

We have previously found that cationic IgG localized to the dermalepidermal junction (DEJ) after intravenous injection in mice. In the present experiment the influence of size of cationic antigens on the deposition of immune complexes at the DEJ was examined. C57B1/6J mice were injected intravenously with cationized 19S human IgM ($19S_{ED}$), or cationized 7S human IgM ($7S_{ED}$), obtained by reduction and alkylation of the $19S_{ED}$. Rabbit antiserum to human IgM was then given intravenously at varied time points and mice were sacrificed 1 hr later. The cutaneous localization of antigen, antibody and mouse C3 was examined by immunofluorescence microscopy:

Deposition of human IgM as antigen (HIgM), rabbit IgG as antibody (RIgG), and mouse C3, in cutaneous vessels (V) and DEJ (D):

Time	$7S_{ED}$ RIgG		$7S_{ED}$ HIgM		$7S_{ED}$ C3		19S RIgG		19S HIgM		19S C3	
	V	D	V	D	V	D	V	D	V	D	V	D
1 h	3+	1+	2.5+	0	1+	0	3+	0	2+	0	1+	0
8 h	4+	2+	2.5+	2+	2+	0	4+	tr	3+	0	2+	0
24 h	4+	2+	2.5+	3+	1.5+	tr	2.5+	0	2.5+	0	tr	0

Blood disappearance kinetics were similar for the $7S_{ED}$ and $19S_{ED}$. Antiserum given alone did not form deposits. These data indicate that size as well as charge of circulating antigens is important in determining the sites of cutaneous formation of immune complexes and that access of antigens to the DEJ is restricted to a size smaller than 19S IgM. Circulating cationic antigens may also require significant time to reach the DEJ.

10. Patients with Bone Marrow Failure Demonstrate Decreased Cutaneous Reactivity to Human C5a. K. YANCEY, L. BIELORY, R. WRIGHT, M. KALINER, N. YOUNG, M. FRANK, AND T. LAWLEY, NIH, Bethesda, MD.

In vivo studies have shown that human C5A is a potent mediator of inflammation in normal human skin. Intradermal (ID) injection of human C5a causes increased vascular permeability, mast cell degranulation, and neutrophil-predominant infiltrates within minutes. Since the relative contribution of these various processes to C5a-induced inflammation in normal human skin is unknown, human C5a was

purified to homogeneity and tested ID (100 and/or 200 ng) in 10 patients with bone marrow failure (BMF) who had very low numbers of neutrophils (PMN's). Reactions to C5a in BMF patients were compared with similar test sites in normal volunteers and skin disease controls. Patients with BMF developed markedly less wheal and flare to C5a than controls ($P < 0.05$). Twenty minutes after ID injection of 200 ng of C5a mean wheal and flare in patients and controls were 9.3/22.6 and 13.8/51.3 mm, respectively. Also, patients with lowest numbers of neutrophils tended to show the least cutaneous reactivity to C5a. Biopsies of C5a test sites in 2 BMF patients showed absence of PMN's in marked contrast to PMN-rich infiltrates in normal volunteers. Avidin-fluorescein and/or Giemsa staining of skin biopsies from 11 patients with BMF revealed dermal mast cells that were indistinguishable from those in control biopsies. ID tests to evaluate reactivity to histamine (2 μg) and morphine (5 μg) in patients with BMF (N = 16) and allergic rhinitis (N = 11) revealed no significant differences in skin test reactions, thus indicating normal vascular and mast cell responses in BMF patients. These *in vivo* studies with C5a show that patients with BMF exhibit a specific, decreased reactivity to human C5a and indicate that PMN's make an important contribution to inflammation initiated by C5a.

11. Plasminogen Activator (PA) and Urokinase Inhibitor (UKI) in the Development of the Epidermis. M. NAKAGAWA, J. FRAKI, K. FUKUYAMA, K. YABE, AND W. L. EPSTEIN, Depts. of Dermatology, University of California, San Francisco, CA, and University of Kuopio, Kuopio, Finland.

PA and UKI are involved in tissue remodeling and cell migration; PA activity is elevated in the epidermis with rapid turnover. We investigated modulation of PA and UKI activities in rat skin at different prenatal stages and after birth. Skin was obtained from Sprague-Dawley rats: 15 and 17 day fetuses and 2 days after birth. Whole fetuses younger than 11 and 13 days also were used. Proteins were extracted stepwise in 20 mM PIPES, pH 6.8, containing 0.14 M NaCl and 100 mM Tris-HCl, pH 8.0, containing 2 M KSCN. By plasminogen-containing fibrin plates we detected PA activity in 2 M KSCN extracts while UKI activity was present in the low salt extract. As summarized below, PA was more abundant in the early stage and decreased gradually; UKI was minimally detectable in <11 day fetuses and increased with time. PA and UKI activities localized in the epidermis after birth.

Activity/ g tissue	Prenatal				2-day-old	
	<11d	13d	15d	17d	Epidermis	Dermis
PA (IU)	3.46 ± 2.2	2.49 ± 1.8	2.36±1.8	1.96 ± 1.3	0.09 ± 0.16	0
UKI (INU)	4.6 ± 5.0	80.7 ± 57	134.9 ± 107	186.6 ± 41	195.6 ± 23	0

Zymograms showed the M_r of PA after birth to be 65.3 ± 4.1 K and 48.5 ± 1.2K but <11 day fetuses contained primarily PA with 65.3 K. The 48.5 PA appeared after 13 days gestation. UKI was more effective against the activity of 48.5K than 65.3K and had a M_r of 33K and 120K as determined by Sephadex G-100 column chromatography. The findings indicate that modulation of PA activities by UKI is associated with histogenesis of the epidermis. PA with different M_rs may have specific biological roles during development.

12. Detection of a Human Lung Mast Cell Chymotryptic Enzyme Which Is Functionally Similar to the Human Skin Chymotryptic Proteinase. BRUCE U. WINTROUB, CAROL E. KAEMPFER, NORMAN M. SCHECHTER, AND DAVID PROUD, VAMC and University of California School of Medicine, San Francisco, CA.

Human mast cells contain releasable serine proteases whose function is unknown. While a tryptase has been recognized and isolated, chymotryptic activities have not been detected. Using an HPLC assay which detects chymotryptic cleavage of the phe_8-his_9 bond of angiotensin I-(AI) to yield angiotensin II (AII), human lung mast cells were examined for chymotryptic activity. Mast cells, purified from human lung by enzymatic dispersion, countercurrent elutriation and Percoll gradient centrifugation, were lysed or challenged with goat antihuman IgE. In multiple experiments, chymotryptic activity was detected in cell lysates, and 35–65% of activity was released by immunologic challenge. S-200 gel filtration of the release supernatant from multiple preparations of purified mast cells demonstrated a single peak of 30–35,000 MW chymotryptic activity which was separated from the 145,000 MW mast cell tryptase as detected by cleavage of ^{3}H-TAMe. Column purified chymotryptic enzyme was assessed for functional identity with the other cell derived human chymotryptic enzymes-leukocyte cathepsin G and skin chymotryptic proteinase. The Km for angiotensin conversion was ~6.6×10^{-5} M, which is similar to that of the skin enzyme but differs from cathepsin. (Inhibition profiles, using soybean and pancreatic trypsin inhibitors showed similar results for the skin and lung enzymes and distinguished these enzymes from cathepsin G. Thus, human lung mast cells contain a chymotryptic enzyme which efficiently converts AI to AII and is functionally similar to the human skin chymotryptic proteinase. While AI conversion was used to detect the lung chymotryptic enzyme, the Km is consistent with a reaction of physiologic importance.

13. Substance P Induced Histamine Release in Human Cutaneous Mast Cells. J. M. EBERTZ, N. S. KETTELKAMP, C. A. HIRSHMAN, H. UNO, AND J. M. HANIFIN, Depts. of Dermatology and Anesthesiology, Oregon Health Sciences University, Portland, OR, and Regional Primate Center, University of Wisconsin, Madison, WI.

Substance P (Sub P) is an undecapeptide found in multiple sites throughout the central and peripheral nervous systems including the small unmyelinated (type C) cutaneous nerve fibers. Previous studies demonstrated that antidromic stimulation results in Sub P release from nerve endings. Sub P stimulated histamine release (HR) from rat mast cells (MC) *in vitro*. Intradermal Sub P in humans produced wheals identical to those induced by histamine. These studies suggest a possible role for Sub P as a link between neurologic structures and cutaneous mast cell mediated reactions. We investigated Sub P induced HR in an *in vitro* preparation of human skin mast cells.

Human foreskins were sectioned into 0.2 mm slices and incubated with buffer for 30 minutes at 37°C to determine spontaneous HR. Following aspiration of the supernate, identical incubations were performed with varying concentrations of Sub P. Residual tissue histamine was obtained by boiling/homogenization of each sample. Histamine was assayed using automated fluorimetry and HR calculated as a percentage of total tissue histamine. Sub P caused dose-dependent HR over a range from 10^{-5} M (1.3 ± 1.1%, r = 10) to 3×10^{-4} M (13.2 ± 2.6%, n = 11). HR was blocked by pretreatment with EGTA and optimal at 3 mM calcium. Naloxone failed to block HR.

Histamine release from skin mast cells by Sub P may be a mechanism for neurologic modulation of poorly understood inflammatory skin conditions.

14. Elastase and Neutral Cathepsins from Human Dermal Fibroblasts in Culture. AMY S. PALLER, ROGER W. PEARSON, PAULA LIZAK, AND DAVID E. SCHWARTZ, Depts. of Dermatology and Biochemistry, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

Fibroblasts from normal adult forearm skin and neonatal foreskin were cultured and examined for their ability to synthesize and secrete elastase and neutral cathepsins. All of the cultures produced elastase, assayed using an insoluble elastin substrate. An enzyme that hydrolyzed the synthetic substrate for elastase, N-succinyl-$(Ala)_3$-p-nitroaniline (SANA), but did not degrade insoluble elastin, was also found. In addition, culture extracts hydrolyzed the synthetic substrate for neutrophil cathepsin G, N-succinyl-Ala-Ala-Pro-Phe-p-nitroaniline (AAPP). Activities against SANA and AAPP were eluted in the same fractions following chromatography of the extract on Sephadex G-75 and DEAE cellulose. The enzyme that cleaved AAPP was distinct from another neutral cathepsin from fibroblast cultures that degraded N-benzoyl-DL-Phe-naphthyl ester (BPNE). Inhibitor profiles indicate that the elastase and SANA degrading activities are due to metalloproteinases. Degradation of BPNE can be inhibited by phenylmethylsulfonyl fluoride (PMSF). Most of the activity of these proteinases was associated with the cells. Minimal enzyme activity was detectable in the culture medium. Although activities of the measured proteinases were detected in all cultures, increased or decreased enzyme activities were not predictably related to passage number of length of serum starvation. Degree of confluence also affected proteinase activities.

15. Plasminogen Activator (PA) Secreted from Activated Macrophages and Its Possible Role in Granulomatous Inflammation. S. IZAKI, T. HIBINO, P. S. HSU, S. TOKAIRIN, M. OHKUMA, AND M. IZAKI, Dept. of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan.

Thioglycollate-stimulated mouse peritoneal macrophages were cultured in order to characterize the secreted PA (mPA). The culture media (Dulbecco's MEM + lactoalbumin hydrolysate without fetal calf serum) were collected every 24 hours, condensed, and partially purified.

An electrophoretic enzymography technique of Heussen and Dowdle (1980) revealed that molecular weight of mPA (45,000 and 24,000) is distinct from that of urokinase-type PA (uPA) (Mr = 55,000 and 33,000) and tissue- type PA (tPA) (Mr = 63,000). An immunoblotting technique showed that antigenic property of mPA is unrelated with both uPA and tPA. A unique property of mPA in substrate specificity for synthetic chromogenic substrates was demonstrated. The mPA showed the highest hydrolytic activity for Ile-Pro-Arg-p-nitroanilide (pNA) (s-2288), the second highest activity for Val-Leu-Lys-pNA (s-2251), and the third for Glu-Gly-Arg-pNA (s-2444), whereas both uPA and tPA showed minimum hydrolytic activity for Val-Leu-Lys-pNA (s-2251).

A PA with identical molecular weight, antigenicity, and substrate specificity was demonstrated in tissue extract of hypersensitivity-type murine lepromas in C57BL/6N mice. This finding was correlated with ultramicroscopic and immunohistochemical observation of murine leprosy that revealed activation and differentiation of macrophages to the secretory-type, and deposition and clearance of tissue fibrin. However, immunologically inactive-type murine lepromas in CBA mice demonstrated no PA activity, suggesting that secretion of mPA in inflammatory tissue plays an immuno-modulatory role during the development of hypersensitivity granulomas.

CONCURRENT SESSION 4D: MATRIX PROTEINS

Senate

Ryohei Ogura and David Woodley, Presiding

1. Fibronectin Mediates Human Keratinocyte Adhesion via the Fibroblastic Cell-Binding Domain. RICHARD A. F. CLARK AND JOY M. FOLKVORD, Depts. of Medicine and Dermatology, National Jewish Hospital and Research Center, Denver, CO.

Fibronectin appears in the provisional extracellular matrix beneath the epithelializing mammalian wounds and may play a critical role in the adherence and movement of the migrating epidermis. In this investigation we demonstrate that fibronectin mediates human keratinocyte adherence *in vitro*, and that the same molecular domain of fibronectin involved in fibroblast attachment is responsible for epidermal adhesion. Keratinocytes were isolated from human neonatal foreskin and cultured *without* fibroblast feeder layers in MCDB 153. Fibronectin promoted the adherence of both primary isolates and second passaged cells in high (1.0 mM) and low (0.1 mM) Ca^{++} to the same degree as heterologous types I and III collagen and to a significantly greater magnitude than either laminin or type IV collagen. Keratinocyte adherence to fibronectin was both time and dose dependent with maximal adherence observed within 1 hr on 100 μg/ml fibronectin adsorbed to 6mm diameter, flat-bottom, bacteriologic-plastic microtiter wells. Polyclonal anti-human fibronectin antibodies specifically inhibited keratinocyte attachment to fibronectin coated surfaces but not adhesion to laminin or heterologous types I and III substrates. Monoclonal antibodies (MoAb) to the fibroblastic cell-binding domain of the fibronectin molecule inhibited keratinocyte adherence to fibronectin while MoAb to the aminoterminus, to the region between the collagen- and cell-binding domains, and to the carboxy-terminus did not prevent adhesion. We take these results to support the hypothesis that fibronectin probably plays a significant role in epidermal cell adhesion and movement across the wound surface during re-epithelialization.

2. Fibronectin Mediated Keratinocyte Migration and Onset of Fibronectin Receptor Function In Vitro. A. TAKASHIMA AND F. GRINNELL, Dept. of Cell Biology, University of Texas Health Science Center, Dallas, TX.

Human keratinocytes, freshly isolated from skin specimens, did not express fibronectin (pFN) receptor function based on assays for cell attachment and spreading on pFN-coated culture dishes or binding and phagocytosis of pFN-coated latex beads. These activities were expressed by cells, however, after 2–4 days of culture. Since the same methods were used for preparing the original cell suspension as well as for harvesting the cultures, it is unlikely that the results reflected removal of pFN receptors during the cell isolation procedures. In addition, similar results were obtained using epidermal explants. After 9 days of culture, the cells migrating out of the explants expressed pFN receptor function while the cells remaining in the central region of the explant did not do so. Analysis of the cell types arising during culturing, based on staining with anti-keratin antibodies and bullous pemphigoid serum, revealed that about 90% of the starting cell population were keratinocytes and 30% were basal cells. By 2 days of culture, 95% were keratinocytes and 70% were basal cells. It was concluded that pFN receptor function was turned on in the basal cell population. In related studies, the role of pFN in epidermal cell migration was analyzed, and it was found that anti-pFN IgG inhibited migration of keratinocytes out of epidermal explants. Addition of preimmune IgG, however, had no effect. It appears, therefore, that pFN is important in all aspects of keratinocyte adhesive abilities; i.e., cell attachment and spreading on substrata, cell binding and phagocytosis of small particles, and cell migration. The expression of pFN receptor function may be a critical activation step necessary for basal cell function during wound healing.

3. Comparative Immunochemical Investigation of Fibronectin and 70K Cell-Spreading Factor in Cell-Matrix Interactions. TOSHIHIRO ADACHI AND OSAMU MIDORIKAWA, Dept. of Pathology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Fibronectin is one of the matrix proteins bound to collagen and heparin, and has an important role for the cell-to-substratum adhesion in vitro. We previously reported that fibronectin is produced from malignant transformed mouse fibroblast cells (m cells) cultured in serum-free medium and it has a biological function to induce cell-spreading when added to so-called reticulum cells (SP cells) in vitro. By the application of this serum-free culture method, we recently detected that fibronectin-removed serum proteins still induce cell-spreading when added to SP cells, and the protein was purified from human serum using various purification procedures. The molecular weight of the protein is apparently 70,000 daltons by sodium dodecyl sulfate polyacrylamide gel electrophoresis. This 70K protein binds to heparin CL-4B, but do not bind to gelatin Sepharose affinity column. Furthermore, antiserum against 70K protein do not cross-react with human fibronectin, and inhibits the cell-spreading effect of the 70K protein in vitro. Immunoenzymatic microscopy showed that the 70K protein localizes in intercellular matrix of the various tissues in vivo. These data suggest that both fibronectin and 70K cell-spreading factor have an important function in cell-surface and matrix interactions in vitro and in vivo, and provide an useful model for the study of cell-surface receptors and transmembrane control.

4. Extracellular Matrices Affect the Secretion and Deposition of Fibronectin by Human Keratinocytes and Their Growth in Culture. M. KUBO, M. KAN, M. ISEMURA, I. YAMANE, AND H. TAGAMI, Depts. of Dermatology and Biochemistry, Tohoku University School of Medicine, and Division of Cell Biology, Research Institute TB Cancer, Sendai, Japan.

Extracellular matrix (ECM) is essential for cell adhesion, migration growth and differentiation. Synthesis of matrix proteins by epithelial cells has been shown to be affected by exterior ECMs. Previously the author reported that human keratinocytes synthesize, secrete and deposit fibronectin (Fn) *in vitro*. Therefore in this paper, we examined whether ECMs have any influence on the secretion and deposition of Fn by human keratinocytes as well as on their growth *in vitro*.

Human keratinocytes were cultured on various ECMs (Fn, laminin, heparain sulfate proteoglycan, type IV collagen, and gelatin)-coated and noncoated (control) dishes in serum-free, low-Ca medium. Localization and deposited amount of Fn were studied immunohistochemically and immunoelectromicroscopically, while secreted Fn in the media was measured by enzyme immunoassay. The growth of keratinocytes was assessed by counting the number of the cells. At early cultures, a small amount of Fn was visualized within cells, and a moderate amount of Fn was demonstrated on the substratum and in the medium of the control dish. As compared with this, a higher amount of Fn was detected intracellularly in the keratinocytes cultured on type IV collagen, on the substratum coated with Fn or gelatin, and in the medium from the culture on type IV collagen or gelatin. An increased growth of keratinocytes was noted only on Fn or gelatin coated dishes. Thus the increased rate of cell growth appeared to correlate with the amount of Fn on the substratum.

We conclude that ECMs modulate the secretion and deposition of Fn by human keratinocytes and thereby control their growth in culture.

5. Neonatal Foreskin Should Not Be Used for the Routine Screening of Dermo-epidermal Junction components. D. T. WOODLEY, R. FALK, M. J. REESE, T. ERNST, W. R. GAMMON, AND R. A. BRIGGAMAN, University of North Carolina Medical School, Chapel Hill, NC.

Neonatal foreskin is often used for screening monoclonal antibodies by indirect immunofluorescence (IIF). Polyclonal antitype IV collagen antibodies detected type IV collagen in the dermo-epidermal junction (DEJ) by week 6 of gestation (Fine, 1984). We compare neonatal

foreskin and adult skin substrate for IIF with DEJ antibodies. Test sera included mouse monoclonal antibodies to the epidermolysis bullosa acquisita antigen (EBA-A) and to type IV collagen, and polyclonal antisera to laminin and to bullous pemphigoid antigen (BPA). ELISA, immunoelectron microscopy (IEM) and western blots defined antibody specificities. IIF was performed on 6–8 μ vertical cryostat sections of human neonatal foreskin or adult skin as substrate. The results are:

Antibody To:	IEM Localization	Neonatal IIF		Adult IIF	
		DEJ	Vessel	DEJ	Vessel
Type IV collagen	LD	±	++++	++++	++++
EBA antigen	LD	++++	−	++++	−
Laminin	LD/LL	+++	++++	+++	++++
BPA	LL	+++	−	+++	−

(+ = positive, − = negative, LD = lamina densa, LL = lamina lucida)

Since the type IV collagen monoclonal antibody reacts strongly with dermal vessels but minimally or not at all with the DEJ of neonatal foreskin, this substrate should not be used for routine screening of antibodies to the DEJ. The reason for the reduced expression of this type IV collagen epitope within the DEJ of neonatal foreskin is unknown.

6. Synthesis of Hyaluronic Acid and Proteoglycans in Differentiating Epidermal Cells Grown on Collagen or Plastic Matrices. STANFORD I. LAMBERG AND VINCENT C. HASCALL, Dept. of Dermatology, Johns Hopkins Medical Institutions, Baltimore, MD, and NIDR, NIH, Bethesda, MD.

We have previously shown that newborn mouse epidermal cells proliferating in 0.05 mM Ca^{++} (LOCA) medium synthesize hyaluronic acid (HA) and that this synthesis is reduced several fold in epidermal cell cultures induced to differentiate by switching to medium that is 1.2 mM Ca^{++} (HICA). In addition, synthesis of glycoproteins (GP) and proteoglycans (PG) increases 2 fold and 20% respectively in the HICA cultures. About 90% of the new PG is heparan sulfate and about 10% is chondroitin or dermatain sulfate.

The study was extended by comparing the synthesis of HA, GP and PG in cultures plated on collagen with that in cultures plated on plastic. The accumulation of newly synthesized glycoconjugates labeled with glucosamine and sulfate precursors in HICA cultures plated on reconstituted, native rat tail tendon collagen increased 250%, compared to 130% for cells plated on plastic. The medium of cultures plated on collagen switched to HICA showed a 3-fold decrease in new HA synthesis. The increase of newly synthesized PG in cultures on collagen switched to HICA was 4-fold greater than with cells grown on plastic. Structural analysis showed no difference in the species of PG made by cells on the two matrices. However, PG was nearly absent from the medium of both LOCA and HICA cells grown on collagen while it was a major component of the medium of cells grown on plastic.

This study shows that collagen stimulates epidermal cells to synthesize glycoconjugates, that the depression of HA synthesis with epidermal differentiation is not matrix dependent and that the matrix does not affect the species of PG synthesized. The mechanisms for the large increase of newly synthesized PGs and their different distribution in the cultures grown on collagen are being studied.

7. Effect of Platelet Homogenate on Glycosaminoglycans Production by Scleroderma and Normal Fibroblasts In Vitro. VINCENT FALANGA, PATRICIA HEBDA, AND WILLIAM H. EAGLSTEIN, Dept. of Dermatology, University of Pittsburgh, Pittsburgh, PA.

Confluent cultures of dermal fibroblasts from the involved skin of scleroderma (SF) and matched skin site of normal control patients (NF) were investigated for the synthesis of glycosaminoglycans (GAG) in response to various concentrations of human platelet homogenate (PH). Experiments were carried out in the presence of 1% and 15% human serum (HS). In the absence of PH, GAG synthesis was higher in SF than NF. An increase in GAG synthesis occurred in 15% HS treated SF and NF, but there was no GAG synthesis increase in 1% HS treated NF. The absolute count of GAG synthesis was always greater in SF than NF. The addition of PH in concentrations higher than 200 mcg/ml led to cell death of both SF and NF. These findings are the first to indicate a difference between SF and NF response to PH.

8. The Extracellular Matrix in Neurofibromas. RAUL FLEISCHMAJER, RUPERT TIMPL, AND MARK G. LEBWOHL, Dept. of Dermatology, Mount Sinai School of Medicine, New York, NY, and The Max Planck Institut für Biochemie, Munich, F.R.G.

Neurofibromas are tumors of neural and mesodermal origin and their main cellular constituents are Schwann cells and fibroblasts. The purpose of this study was to characterize the extracellular matrix of these tumors. Affinity-purified rabbit antibodies were used against: type I, III, IV and VI collagens; type I and III procollagens; laminin, nidogen and fibronectin. These antibodies were used for immunofluorescence and immunoelectron microscopy, and immunoblotting. The most striking feature was an extensive network of basement membranes, mostly around Schwann cells. The lamina densa contain type IV collagen, laminin and nidogen. Laminin and nidogen was extracted with 0.5 M NaCl and by electrophoresis showed chain and fragment patterns similar to those described previously in basement membrane of mouse sarcoma. Collagen IV and VI were solubilized by limited proteolysis and identified by rotary shadowing. Interstitial regions of the tumor contained cross-striated collagen fibrils which were composed of collagen type III (diameter 20–30 nm) or collagen type I (diameter 40–50 nm). Collagen fibrils thicker than 80 nm were not found. The interstitial regions also contained collagen VI as a fine filamentous network near cells and between collagen fibrils. Deposits of fibronectin were rather small and showed a scattered distribution. This study shows that Schwann cells contribute significantly to the matrix of neurofibromas which appear as suitable models for the study of basement membranes of neural-ectodermal origin.

9. Morphological and Biochemical Analyses of Hyaline Substances Produced in the Stroma of Trichilemmoma. AKIRA UMEZAWA, TAMOTSU KANZAKI, AND HITOO IWASE, Depts. of Dermatology and Biochemistry, Kitasato University School of Medicine, Sagamihara, Japan.

So-called hyaline substances are often observed in the stroma of adnexal tumors. However, there is no precise report concerning morphological and biochemical studies of the hyaline substances. We have analyzed these substances morphologically and biochemically.

After inoculating our established trichilemmoma cell line cells in nude mice, hyaline substances were formed in the stroma as observed in the original tumor. Although these hyaline substances appeared to be collagen fibers light and electron microscopically, they were quite different from normal collagen fibers. Because, these fibers were thickened (2 times than normal) with disapperance of regular cross-banding patterns in the most parts when fibers were examined by a negative stain method. Biochemically, we performed amino acid analysis by amino acid analyzer and sugar analysis by gas chromatography after obtaining acetic acid soluble, pepsin soluble and pepsin insoluble fractions. Hyaline substances were found to be collageneous substances by amino acid analyses and found to contend extremely higher amount of glucose in the pepsin insoluble fraction, i.e., 798 vs. 54 in glucose and 80 vs. 57 in galactose n moles/mg dry weight in hyaline vs. normal dermal collagen. In order to exclude the possibilities of the contamination of glycogen in this fraction, the fraction was treated with α-amylase. Release of a very small amount of glucose was observed (less than 10% in total glucose level).

These results suggest that the hyaline substances are highly glycosylated collagen fibers and undetermined glycosylated substances associated with collagen fibers.

10. Anchoring Fibril Staining in Cultured Epithelial Cells. L. A. GOLDSMITH, A. T. LANE, AND P. E. MCCOON, Dermatology Unit, University of Rochester School of Medicine and Dentistry, Rochester, NY.

AF1 is a murine monoclonal antibody which stains human anchoring fibrils. In these studies, AF1 stains the cytoplasm of ME-180 cells, a human cervical carcinoma cell line, and the basal cell cytoplasm of cultured human epidermal cells. Cultured ME-180 cells were unfixed, or fixed in 10% formalin for 10 min., permeabilized with .1% saponin for 2 min., blocked with 20% goat serum, washed and incubated in AF1. Murine anti-HLA (W6/32) and pemphigus human serum of 1/20 dilution were + controls and other monoclonal antibodies which do not react with epidermis were − controls. On unfixed cells HLA and pemphigus gave a thin ring pattern. Other antibodies, including AF1 were −. On fixed and permabilized cells AF1 gave + diffuse cytoplasmic staining while HLA and pemphigus were still + with a ring pattern. Human foreskin epidermal cells were cultured for 35 days. The stratified cells were washed twice with serum-free media and incubated for 1 hr. at 37°C with 1.2 units/ml dispase. The epidermal cells completely detached from the substrate and were washed with serum-free media,

embedded in OCT, frozen and sectioned at 4 μm. AF1 intensely stained the lower half of basal cell cytoplasm. Similar cytoplasmic staining is present during late fetal life and after freeze-thawing of foreskin epidermis. Pemphigus produced its characteristic intercellular patterns and 2 other monoclonal antibodies were −. These findings suggest that the anchoring fibril antigen is present intracellularly in epidermal and ME-180 cervical cells and its secretion to a completely extracellular location doesn't occur in cultured keratinocytes. The effect of pharmacological agent, Tunicamycin and studies in recessive dystrophic epidermolysis bullosa should allow identification of the control mechanisms for the secretion of AF1.

11. Basal Cell Carcinomas Grown in Nude Mice Produce and Deposit Fibronectin in the Extracellular Matrix. RONALD E. GRIMWOOD, CHARLES F. FERRIS, LARRY E. NIELSEN, J. CLARK HUFF, AND RICHARD A. F. CLARK, Depts. of Medicine and Clinical Investigation, Fitzsimons Army Medical Center, and Dept. of Dermatology, University of Colorado School of Medicine and National Jewish Hospital, Denver, CO.

Epidermal cells *in vitro* produce and deposit fibronectin (FN) in the pericellular matrix. Such FN production by epidermal cells may be involved *in vivo* in reepithelialization during wound healing, tissue morphogenesis, and growth of epithelial tumors. The purpose of this study is to examine in a common human epithelial tumor, basal cell carcinoma (BCC), grown on the nude mouse, whether the FN within and surrounding the tumor is the product of the BCC or whether it is the product of the stroma.

Five solid BCC's were transplanted to nude mice. The growing BCC tumors were removed after 60 days, snap frozen, and sectioned on a cryostat. The presence of BCC was verified microscopically, and the pesence of mouse stromal cells surrounding the human tumor was verified by the Hoescht DNA stain, which differentiates mouse from human nuclei. Sections of all five BCC's were stained by immunofluorescence and immunoperoxidase techniques with antibodies to bullous pemphigoid antigen (BP), laminin (LM) and FN. BP and LM were present at the basement membrane zone (BMZ) of all tumor lobules. FN staining was present along the BMZ, within the tumor lobules, and in the surrounding stroma. Antibodies to human FN were passed over a mouse FN affinity column to produce antibodies which reacted with human FN but not mouse FN. Antibodies specific for human FN continued to demonstrate FN along the BMZ and within the tumors, while staining in the surrounding stroma was absent. Human BCC cells therefore synthesize and deposit FN *in vivo* in the extracellular matrix. We speculate that this FN matrix may facilitte growth of BCC in this model.

12. The Interaction of Keratinocytes and Extracellular Matrix. III. Characterization of the Cell Adhesion Process and Involvement of Membrane Glycoprotein(s). TAMARA BYK, SARA KUNIN, JUDIT ROSENSHTOCK, DALIA GINSBERG, BENO MICHEL*, AND YORAM MILNER, * Dept. of Dermatology, Case Western Reserve University, Cleveland, OH, and Dept. of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel.

The adhesion process of guinea-pig keratinocytes to extracellular matrix secreted on culture plates by keratinocytes (KEM) was investigated. The process, performed on plastic and KEM coated dishes, was found to be metabolic energy dependent (inhibited by NaN_3), temperature dependent and mediated by the cell cytoskeletal system (inhibited by colchicine and cytochalasin B). About 80–90% of cells from a "young" cell population, isolated by differential isopycnic centrifugation in Percoll gradients, attached onto extracellular matrix. On the other hand, 20% of the mature cells and cells from aged animals attached onto matrix covered plates. In order to find the proteins involved in cell-substrate interactions and their variations both with age and maturation, we used lectin and protease treatments of intact cells as probes for cell attachment. Concanavalin A and wheat germ agglutinin, but not peanut lectin, inhibited cell attachment on plastic or KEM in a dose-response curve matching the binding of lectin to cell surface. Treatment of cells with trypsin, chymotrypsin and pronase led to inhibition of attachment of 50%, 40% and 80%, respectively, but was less than 40% when the incubation continued during 14 hours (resynthesis of surface glycoproteins). Proteolysis of the cells led to only 20–25% inhibition of attachment when tested on KEM, indicating a substitution of cell surface glycoprotein by KEM component(s). Plasma membranes isolated from proteolysed cells showed, in SDS-PAGE, a decrease in glycoprotein bands in the M.W. range of 120–250 Kilodaltons—possible adhesion proteins.

13. Reappearance of Seven Basement Membrane Antigens in Primate Skin Following Split-Thickness Wound Induction. JO-DAVID FINE, DALE A. REDMER, AND ARNOLD L. GOODMAN, Depts. of Dermatology and Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL.

Recently it has been shown that laminin, type IV collagen, and fibronectin are the first detectable basement membrane antigens in developing human fetal skin, followed by epidermolysis bullosa acquisita antigen (EBA), bullous pemphigoid antigen (BPA), and KF-1 antigen. In contrast, BPA precedes laminin and type IV collagen in appearance in swine skin following split-thickness wounding. In order to evaluate this apparent dichotomy, we produced split-thickness wounds in two cynomolgus monkeys and followed reconstitution of epidermis and basement membrane by immunofluorescence technique using antibodies directed against BPA, laminin, type IV collagen, cicatricial pemphigoid antigen (CPA), fibronectin, EBA, and KF-1. Biopsies were obtained on days 3–8, 10, 11, 14, and 18; re-epithelialization was undetectable prior to day 4. On day 4, laminin, type IV collagen, and fibronectin were focally present under the epithelium closest to the periphery of the wound although absent underneath the epithelial advancing edge. By days 6, 8, and 10, BPA, EBA, and CPA were first detectable, respectively. KF-1 was undetectable until day 11; however, uniform expression of this antigen was not noted until day 18. These data suggest that the sequence of basement membrane antigen reconstitution following split-thickness wound induction in primate skin parallels the ontogeny of these antigens in human skin. Futhermore, these data suggest, despite the findings in swine skin, that bullous pemphigoid antigen may not play a critical role in epithelial cell migration across wounded primate skin.

14. Immunohistological Demonstration of Basement Membrane Components in Granuloma Extracellular Matrix of Murine Schistosomiasis. MASAYUKI NISHIMURA, MASAO HAYASHI*, MASAKAZU ASAHI†, AND HIROMU KOHDA, Division of Dermatology, Saga Medical School, Saga, Japan, * Institute of Biological Sciences, Tsukuba University, Ibaraki, Japan, and † Dept. of Dermatology, Kyushu University, Fukuoka, Japan.

Schistosome egg granulomas which develop in mice with an intact thymus (nu/+) are large and well organized, while those in athymic (nu/nu) mice are small and poorly organized. In this regard, macrophages are reported, in vitro, to express cell surface fibronectin and laminin which mediate several important cellular activities such as attachment, differentiation etc. (Exp Cell Res 143: 475, 1983). To investigate a possible role of extracellular matrix molecules in the development of granulomatous inflammation, we analyzed and compared extracellular matrix of schistosome egg granulomas of nu/+ and nu/nu mice. Cercariae of Puerto Rican stain Schistosoma mansoni were subcutaneously injected into nu/+ and nu/nu mice of BALB/c background, and 11 weeks later granulomatous livers were removed. Specimens were cut into 3 pieces and processed separately for fluorescence, light, and electron microscopic studies. Indirect immunofluorescence was carried out using specific antibodies directed against fibronectin, laminin, heparan sulfate proteoglycan (HSPG), and type II collagen. Granulomas were composed mainly of stimulated macrophages and epithelioid cells. In nu/nu mice, granulomas were much smaller and less well organized than in nu/+ mice. Fibronectin and laminin codistributed with HSPG and type IV collagen in nu/+ mice. However, only fibronectin and type IV collagen were detected in nu/nu mice. The results suggest that laminin and HSPG play an important role in the organization of macrophages in granulomatous inflammation. Expression of laminin and HSPG in the granuloma extracellular matrix might be a thymus dependent phenomenon.

15. The Enhancement of Epidermal Cell Attachment by Basement Membrane Entactin. S. P. ALSTADT, P. A. HEBDA, A. E. CHUNG*, AND W. H. EAGLSTEIN, Depts. of Dermatology and * Biological Sciences, University of Pittsburgh, Pittsburgh, PA.

A Matrix of Laminin and Entactin, extracted from the basement membrane of a mouse endodermal cell line (M1536), has been used to assay the contribution of Entactin to epidermal cell attachment. We have previously shown that this Matrix enhances epidermal cell attachment and proliferation when compared to commercial Laminin or plastic alone (J Invest Dermatol, July 1984, same authors). A suspension of epidermal cells in serum-free medium was allowed to attach to a substratum of Laminin, Matrix, or albumin-pretreated plastic. The number of attached cells was then determined by a hemacytometer count. Varying the amount of Laminin or Matrix used as a substratum

showed that both enhanced attachment to the same maximum, but Matrix was effective in lower amounts than Laminin ($p < 0.05$). Antibody binding was used to block cell attachment to the Entactin in the Matrix. Cell attachment was assayed after substratum pretreatment with Affigel Blue-purified anti-Entactin antibody. Increasing amounts of anti-Entactin decreased attachment to Matrix in a concentration-dependent manner, eventually falling to the same level seen with Laminin. Anti-Entactin had no effect on cell attachment to Laminin or to the plastic control, and nonspecific rabbit IgG had no effect on any group. These results indicate that the ability of Matrix to enhance cell attachment above the level seen with Laminin is probably due to the presence of Entactin. Since cell attachment is important in migration and mitosis, Entactin may be important in the normal processes of epidermal wound healing and cell differentiation.

Sunday, May 5, 1985

7:30 AM–8:00 AM
Business Meetings:
SID—William Epstein, presiding Presidential Ballroom
JSID—Nobuyuki Mizuno, presiding Congressional
8:00 AM–10:00 AM Presidential Ballroom

PLENARY SESSION III
Akira Ohkawara and Peyton Weary, Presiding

1. Relationship Between Keratinocyte and Lymphocyte Derived Interleukin 3. THOMAS A. LUGER, ANDREAS KÖCK, AGATHA URBANSKA, MICHEL COLOT, AND MICHEAL MICKSCHE, Dept. of Dermatology II and Institute of Applied and Experimental Oncology, University of Vienna, Vienna, Austria.

Interleukin 3(IL3) initially was identified as a lymphocyte derived mediator. Like mast cell growth factor (MCGF) and colony stimulating factor (CSF)IL3 stimulates the proliferation of multipotential hemopoetic stem cell lines as well as mast cell like cell lines. Since epidermal cells recently have been shown to release an IL3 like cytokine (ECIL3) the present study was performed to investigate whether ECIL3 is distinct from lymphocyte IL3. For the detection of IL3 activity factor dependent cell lines (32 DCL, FDCP) were used. Supernatants from freshly isolated murine EC as well as from a transformed keratinocyte cell line (Pam 212) contained significant levels of ECIL3 activity. ECIL3 synthesis significantly was enhanced by addition of mitogens, lipopolysaccharides, phorbol esters and Interleukin 2. When EC were depleted of Langerhans cells by treating with anti Ia antiserum the supernatant ECIL3 activity was not diminished, suggesting that keratinocytes are the major source of ECIL3. Furthermore ECIL3 was chemotactic for mast cell like 32 DCL cells. Biochemical characterization using high performance liquid chromatography (HPLC) showed that ECIL3 has a molecular weight of approximately 3o Kd and upon chromatofocusing exhibited 3 isoelectric points (pI 7.8, 7.4, and 7.1). ECIL3 activity eluted as a single peak of activity from reverse phase HPLC. Thus ECIL3 biochemically was not separable from lymphocyte IL3. When compared to normal rabbit serum an anti IL3 antiserum blocked (Dr. J. Ihle, FCRF, Frederick, MD) ECIL3 mediated proliferation of 32 DCL œlls in a dose dependent manner, suggesting that both ECIL3 and IL3 share antigenically similar domains. Therefore through the synthesis of ECIL3 and ETAF, keratinocytes may have a regulatory function during the development of inflammatory diseases.

2. Cellular Sources of Epidermally Derived Signals in Contact Hypersensitivity (CH). J. W. STREILEIN, S. SULLIVAN, P. R. BERGSTRESSER, AND R. TIGELAAR, Dept. of Microbiology/Immunology, University of Miami, Miami, FL, and Dept. of Dermatology, UTHSC at Dallas, Dallas, TX.

In the induction of CH by epicutaneous application of hapten, antigen-specific signals appear to arise from the epidermal compartment. In order to identify the cellular sources, purified Langerhans cells, Thy 1+ dendritic cells, and keratinocytes were prepared from CBA mouse skin via trypsin dissociation, ficoll gradient separation of viable cells, and fluorescence activated cell sorting. Each cell type was derivatized in vitro with trinitrobenzene sulfonate and inoculated (6000/recipient) into syngeneic mice that were assayed for induction of CH and for specific unresponsiveness. The following results were obtained:

Hapten-derivatized cell type	*Route of injection*	*Type of response*
Langerhans Cells	Subcutaneous	Contact Hypersensitivity
Langerhans Cell	Intravenous	Contact Hypersentivity
Thy 1+ Dendritic Cell	Subcutaneous	None
Thy 1+ Dendritic Cell	Intravenous	Specific Unresponsiveness
Keratinocyte	Subcutaneous	Contact Hypersensitivity
Keratinocyte	Intravenous	None

Thus, following hapten-derivatization, an immunogenic signal arises from Langerhans cells, but a down-regulating signal arises from Thy 1+ cells. Keratinocytes serve only to carry hapten to second order antigen-presenting cells in draining lymph nodes. Since epicutaneous application of hapten typically produces CH, not unresponsiveness, the LC-derived signal usually dominates, presumably by virtue of its capacity to act both locally and centrally, whereas the Thy 1+ cell-derived signal can only act centrally.

3. The Role of Lysosomal Protease in Junctional Blister Formation in Epidermolysis Bullosa Letalis. M. MATSUMOTO, K. HASHIMOTO, AND A. OHKAWARA, Depts. of Dermatology, Wayne State University, Detroit, MI, VAMC, Allen Park, MI, and Asahikawa Medical College, Asahikawa, Japan.

Our previous data (J Invest Dermatol 82:392, 1984) had shown that blister fluid from epidermolysis bullosa letalis induced dermal-epidermal separation at lamina lucida. Recently, we could add one more case, and further studies on biochemical and ultrastructural aspects were performed. The previous observations could be confirmed with this new case: Normal skin incubated with the blister fluid showed lamina lucida separation in 24 hrs. The activity of blister fluid could be inhibited by low (pH4) and high (pH9) pHs of the media, by serine protease inhibitors (aprotinin, soybean, PMSF) or universal protease inhibitor (α-2 macroglobulin). Other types of inhibitors (EDTA, antipain n-methyl-maleimide) did not work. Boiling and dialyzation (MW cut off: 2,000) did not affect the activity but a prolonged autoclaving destroyed it. Electron microscopy of the epidermis covering the early blister revealed a number of membrane-bound dense bodies and multivesicular bodies in the basal cells. These were discharged into the blister cavity and some were found among degenerating collagen fibers. Normal skin incubated with blister fluid for 12 hrs. showed similar bodies and their discharge into the early separation; thus natural process of blister formation could be reproduced. Non-blistered patient's skin did not have them. Each experiment was checked with proper controls. It was concluded that the junctional separation in EB letalis is caused by lysosomal serine protease. The blister fluid may contain active proteases or protease activator.

4. Expression of the BPV E6 Transforming Gene via Mammalian and Bacterial Vectors. ELLIOT J. ANDROPHY, JOHN T. SCHILLER, AND DOUGLAS R. LOWY, Laboratory of Cellular Oncology, NCI, Bethesda, MD.

DNA sequence analysis of the Bovine (BPV) and Human Papillomavirus (HPV) genomes reveals the presence of several open reading frames (ORF) which may encode the non-structural proteins of these viruses. Previous genetic studies have demonstrated that the region encoding the BPV E6 ORF can induce tumorigenic transformation of mouse C127 cells in vitro. Additionally, the HPV E6 ORF is preferentially conserved in human cervical carcinoma cell lines. However, low level expression of PV transforming genes and lack of antibodies directed against their gene products have hampered studies of these viral encoded proteins.

We have taken two approaches to characterize the BPV E6 protein. To facilitate increased expression in mammalian cells, we have inserted the BPV E6 gene into a retroviral DNA vector. The presence of the E6 gene in the resulting recombinant retrovirus enables it to transform C127 cells; these cells express high levels of E6 RNA because the E6 gene is under retroviral control. To synthesize very large amounts of the protein encoded by this gene, we have cloned the BPV E6 ORF into a bacterial expression vector. In this construction, the E6 gene is under the control of a thermally inducible, strong bacterial promoter of RNA transcription. At 42°C, the bacteria synthesize the predicted 18 kd protein, which accumulates to over 10% of total bacterial protein. This protein was isolated and used for immunization of rabbits, resulting in the production of antibodies that recognize the E6 protein.

These mammalian and bacterial expression vectors enable the isolation of virtually limitless amounts of E6 protein, production of

antibodies to it, and systems for determining the biochemical properties of the E6 protein.

5. Experimental Systemic Photoallergy. PAULA GIUDICI AND HENRY C. MAGUIRE, JR., Hahnemann University School of Medicine, Philadelphia, PA.

We have induced photoallergy in mice to systemically administered drugs, specifically sulfanilamide (Sulfa) and chlorpromazine (CPZ). Mice were photosensitized to systemic sulfa or CPZ by intraperitoneal (IP) administration of drug followed by UVB and UVA irradiation (UVR) of shaved flank skin, on two consecutive days. Control mice received IP drug with no irradiation. In some experiments cyclophosphamide (Cy) pretreament was administered as an immunoadjuvant. All animals were photochallenged five days later with IP drug followed by UVA irradiation of one ear. Ear swelling and erythema developed 24 hours later in mice that had been previously immunized with drug and UV light. Control animals showed no reactions, thereby demonstrating that this was not a phototoxic reaction. A typical experiment with Balb/c X A mice is outlined:

Group	Day 2	Day 0, 1 Photosens.	Day 5 Photochall.	Day 6 Left	Day 6 Right
I (6)	Cy 150 mg	IP Sulfa, UVR	IP Sulfa, UVA L ear	6.0*	−0.5
II (6)	Cy 150 mg	IP Sulfa, UVR	IP CPZ, UVA L ear	−0.7	−0.7
III (6)	Cy 150 mg	IP Sulfa only	IP Sulfa, UVA L ear	−0.8	−1.0

* Increase in ear thickness (1×10^{-2} mm); I > II, III (left) p = .001. We have adoptively transferred systemic sulfanilamide photoallergy to naive recipients with lymph node cells (5×10^7 IV) from photosensitized donors. Many case reports describe patients on systemic drugs who develop, in a photodistribution, cutaneous reactions that are presumed to represent photoallergy. Our experiments offer the first objective proof that systemic drug followed by UV exposure of the skin can result in a cell-mediated photoallergic dermatitis.

6. Mechanism(s) Regulating Selective Growth Inhibition and Disintegration of Melanoma Cells by 4-S-Cysteinlphenol and Related Compounds. KOWICHI JIMBOW, SHOUSUKE ITO*, KAZUO MAEDA, SHUNSUKE MIURA, HIROYUKI TAKAHASHI, TSUTOMU KASUGA†. AND KIICHI ISHIKAWA‡, Dept. of Dermatology, Sapporo Medical College, Sapporo, *Dept. of Chemistry, School of Medicine, Fujita-Gakuen University, Toyoake, † Dept. of Pathology, Tokyo Medical and Dental University, Tokyo, and ‡ Dept. of Biochemistry, School of Medicine, Yamagata University, Yamagata, Japan.

We have shown that 4-S-cysteinylphenol (CP) and cysteaminylphenol (CAP) inhibit the growth of malignant melanoma (MM) in experimental mice. In this study, we clarified the mechanism(s) regulating the selective growth inhibition and disintegration of MM cells by 4-S-CP and CAP. In experiments with incorporation of H^3-4-S-CP into MM and normal organs, and with autoradiography of whole body and cultured cells, 4-S-CP was selectively accumulated in MM and its incorporation was grossly in parallel to the degree of melanin synthesis. In kinetic studies with mushroom tyrosinase, 4-S-CP and CAP were the substrates of tyrosinase while their 2-S-isomers were not, Km of 4-S-CP, CAP and tyrosine being 0.093, 0.093 and 0.33 and Vmax being 12, 5.7 and 1.8 respectively. Elementary analysis with incubation of 4-S-CP with cysteine and tyrosinase indicated that 4-S-CP is converted to cysteinylcatechol and, then, reacts with cytoplasmic SH-containing enzyme(s) to form dicysteinylcatechol, thus becoming highly toxic. In experiment with incorporation of leucine into microsome and cytosol fractions, 4-S-CP did not inhibit the protein synthesis of MM cells by itself compared with 4-S-cysteinylcatechol, % inhibition being of 5.7 and 33.4 respectively. We suggest that 4-S-CP and CAP are selectively accumulated in MM cells and are not toxic by themselves, but become toxic after conversion to catechols through tyrosinase, thus 4-S-CP and CAP being ideal sources for rational chemotherapy of MM.

7. Molecular Organization of a DNA Segment Containing Human Keratin Genes. E. KLINGE, I. M. FREEDBERG, AND M. BLUMENBERG, Depts. of Dermatology and Biochemistry. NYU Medical Center, New York, NY.

Keratins, the intermediate filaments of epithelial cells, consist of about 20 different protein subunits separable into two families. These two families are themselves members of a superfamily that also includes the intermediate filament proteins of nonepithelial cells, e.g. desmin, vimentin. Each keratin protein is encoded by its own specific mRNA and presumably has its own coding region in the human genome.

As a portion of our studies aimed at elucidating the organization of the keratin genes, we have screened a human genomic DNA library with a number of probes containing human and sheep keratin cDNAs coding for members of each of the two families. Most of the isolated segments of human genomic DNA hybridize with only one of the two types of keratin probes, indicating that they contain coding sequences for only one of the two types. Five per cent of the isolates hybridized with both types. In one case, a 25 kilobase region of DNA was found to contain both types of keratin-specific sequences in three separate segments. Since other studies indicate that the homology between characterized members of the two families is limited, we believe that this region of the human genome contains more than one keratin gene. This means that at least some keratin genes are tightly linked in the human genome. Rigid proof of our hypothesis awaits completion of the sequencing studies currently underway.

8. Preservation of Cholinergic and Adrenergic Responsiveness in Cultured Eccrine Secretory Cells. H. YOKOZEKI, K. SAGA, AND K. SATO, Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

We wished to determine whether sweat gland cells grow in culture and retain their glandular function for a prolonged period of time. Eccrine sweat glands were dissected out from biopsy skin specimens of the rhesus monkey palm and subjected to collagenase digestion to prepare dispersed cells. Approximately 10×10^3 cells were plated in a plastic well precoated with collagen gel and containing a mixture of Ham's F-12 and Dulbecco's MEM (1:1). Secretory cells grow better on tyep 6 than on type 1 collagen but the ductal cells prefer type 1 collagen. Plating efficiency was higher than 70% in the secretory cells. Among different combinations of various common growth factors, a combination of monkey serum (10%) and epidermal growth factor (10 ng/ml) yielded the best growth stimulation in both the ductal and secretory cells. Thus far, secretory cells have survived up to 22 passages over 7 weeks. Since in eccrine sweat glands the sweat secretory activity closely parallels $^{14}CO_2$ production from 6-^{14}C-glucose, we determined CO_2 production as a measure of cholinergic (MCH) and beta adrenergic (ISO) responsiveness in the cultured secretory cells. In fact, both MCH- and ISO-stimulated $^{14}CO_2$ production of the cultured cells is also found to be a saturation function of agonist concentrations and is inhibited by the respective antagonists and by 10^{-5} M ouabain. MCH- or ISO-stimulated $^{14}CO_2$ production gradually decreases over a 7-week period to 30% of control. Stimulation of cultured cells with MCH for 30 min every other day for 2 weeks doubled MCH-induced $^{14}CO_2$ production. We conclude that cell culture of the sweat gland will provide a useful model system for studies of glandular functions in vitro.

9. Modulation of Antigen Presenting Cell Function by In Vitro Administration of Anti-Ia Antibodies. W. ABERER, A. M. KRUISBEEK, S. SHIMADA, AND S. I. KATZ, National Cancer Institute, NIH, Bethesda, MD.

The in vitro activation of T cells by a variety of antigens can be inhibited by administration of anti-Ia antibodies at the time of antigen priming. This inhibition has been suggested to be due to the temporary loss of Ia molecules from Ia-bearing antigen presenting cells (APC) in the spleen. The purpose of this study was to determine whether the intraperitoneal administration of anti-Ia antibodies affects the APC function of epidermal Langerhans cells (LC) in vitro. BALB/c, C3H and (C3H × B10.D2) F_1 mice were injected with anti-Ia containing ascites. Epidermal cell (EC) and spleen cell (SC) suspensions were prepared and assayed for APC function using T cell clones specific for soluble protein antigens or TNP as indicator cells. A single injection of antibody completely abolished the APC function of SC but had no effect on LC using any of the antigens tested. In a representative experiment anti-Ia treatment reduced the capacity of SC to induce T cell proliferation in 3 antigen specific clones by 85 to 99% whereas it reduced the capacity of LC by maximum of 8%. Injections of anti-Ia on three consecutive days also did not significantly affect LC function. In order to determine whether the anti-Ia antibody bound to the LC we prepared epidermal sheets after anti-Ia injection and found that the anti-Ia antibodies had indeed bound in vivo. The density of LC in these sheets was not affected by anti-Ia administration. Thus, in vivo anti-Ia administration appears to differentially affect APC from spleen and

epidermis. The abrogation of splenic but not LC APC function with anti-Ia antibodies will facilitate the dissection of the relative contributions of LC and other antigen presenting cells in the generation of cutaneous immune responses.

10. Specific Binding of Fibronectin to Epidermolysis Bullosa Acquisita Antigen. D. T. WOODLEY, E. J. O'KEEFE, R. A. BRIGGAMAN, M. J. REESE, AND W. R. GAMMON. Dept. of Dermatology, University of North Carolina School of Medicine, Chapel Hill, NC.

The epidermolysis bullosa acquisita (EBA) antigen, a major component of cutaneous basement membrane (CBM), is localized within and immediately below the lamina densa and is recognized as two proteins in extracts of CBM: a major 290 kd band and a minor 145 kd band. To determine whether these proteins have affinity for the extracellular matrix proteins, fibronectin and laminin, CBM proteins were extracted from de-epidermized human skin, separated on 5% polyacrylamide gels and electrophoretically transferred to nitrocellulose paper. Identical nitrocellulose strips containing immobilized EBA antigen and other matrix proteins were incubated with EBA sera, ^{125}I-labeled laminin, or ^{125}I-labeled fibronectin and washed extensively. Antibody binding was detected with ^{125}I-labeled staphylococcal protein A and fluorography. The binding of ^{125}I-labeled fibronectin or laminin to proteins immobilized on the strip was detected by fluorography. Antibodies in EBA sera bound to the 290 kd and 145 kd proteins. ^{125}I-labeled fibronectin bound to these same two proteins even in the presence of 0.25M NaCl and 3% NP-40 but was inhibited by 250 μg/ml of gelatin (but not by similar quantities of unrelated proteins). In contrast, ^{125}I-labeled laminin did not bind to either of the EBA bands. ^{125}I-labeled fibronectin also bound to dermal and purified collagens I, IV, and V, but not to laminin, albumin, immunoglobulin or other dermal proteins. We conclude that fibronectin, via its gelatin binding domain, his affinity for EBA antigen, which may have collagenous domains. Affinity between EBA and fibronectin may be important in maintaining epidermal-dermal adherence.

10:00 AM–12:00 PM Second Floor

POSTER SESSION II

Authors at Posters for Discussion

1. Relationship Between Naturally Occurring T Lymphocytotoxic Antibodies in Viral and Related Diseases and Their Target T Lymphocyte Subsets. HIROSHI HOSOKAWA, SUMIHITO HORIO, AND YASUO ASADA, Dept. of Dermatology, Kansai Medical University, Moriguchi, Japan.

Naturally occurring T lymphocytotoxic antibody (NTA) detected in SLE patients appears to be preferentially directed against the suppressor subset of T cells. However, there is few reports on relationship between NTA in patients with viral and related diseases and the target T cell subsets. This study was undertaken to determine whether NTA in viral and related diseases were directed against a particular subset of T cells.

Sera were obtained from 3 patients with viral exanthema, 2 with pityriasis rosea, 3 with nasopharyngeal carcinoma (NPC), 5 with SLE and 10 healthy adults. In our previous studies we found that these sera without healthy adults contained NTA which were mostly in IgM subclass and cold reactive. After treated with sera and rabbit complement, T cells were counted by monoclonal antibodies against human T cell subsets (OKT-4, 8) and compared with those of healthy controls.

It was demonstrated that killing ratios of OKT-4/OKT-8 by NTA from 2 patients with viral exanthema, 2 with pityriasis rosea and 4 with SLE were increased compared wtih the normal OKT-4/OKT-8 ratio. On the contrary, low killing ratios of OKT-4/OKT-8 by NTA from 3 patients with NPC were shown. These results indicate that NTA present in viral exanthema, pityriasis rosea and SLE may be preferentially directed against OKT-8 (suppressor/cytotoxic) positive T cells, whereas those of NPC may be preferentially directed against OKT-4 (helper/inducer) positive T cells.

2. Natural Killer (NK) Cell Activity and Subpopulation of Peripheral Blood Lymphoid Cells (PBLCs) in Behçet's Disease. FUMIO KANEKO, YOSHIMASA TAKAHASHI, RYUICHI MURAMATSU, YUSHO MIURA, AND TOMONORI MINAGAWA*, Depts. of Dermatology and *Microbiology, Hokkaido University School of Medicine, Sapporo, Japan.

In order to understand the immunological condition of patients with Behcet's disease, we have studied NK cell activity and analysed the subpopulation of PBLCs of 18 patients with active and inactive stage of the disease. The NK cell activity showed a significantly low % of cytolysis of ^{51}Cr labelled K 562 cells, 11.6 ± 7.3 (mean ± SD) ($p < 0.001$), when it was compared with in the inactive stage and with normal controls. On the other hand, the population of NK cells carrying Leu 7 was 24.9 ± 7.3% ($p < 0.05$) out of 2,242 ± 692 PBLCs/mm^3 in the active stage, which was an increase in number, while the number of helper/inducer T cells carrying Leu 3a was reduced. Since it has been suggested that the clinical stages of Behçet's disease causes the serum levels of spontaneous gamma interferon (IFN) to fluctuate from low (the active stage) to high (the inactive stage), we therefore added alpha IFN to the culture media of PBLCs to increase NK cell activity. Although the augment-ratio of NK cell activity was improved, the increase was still insufficient.

These immunological conditions suggest that some factors which activate NK cells in the clinically active stage of the disease are lacking in association with the reduction of helper/inducer T cells, and that the increased number of NK cells in PBLCs of the patients correlates to the lesional infiltration of NK cells.

3. Circulating T and B Cell Abnormalities in Cutaneous Lupus Erythematosus (LE). P. KIND, P. E. LIPSKY, AND R. D. SONTHEIMER, Depts. of Dermatology and Internal Medicine, UTHSCD/Southwestern Medical School, Dallas, TX.

In order to further clarify the relationship between cutaneous LE and systemic LE (SLE) we have examined discoid LE (DLE) and subacute cutaneous LE (SCLE) patients (pts) for the types of circulating T & B cell abnormalities that have been reported in unselected SLE pts. Circulating T & B cell markers were analyzed and circulating immunoglobulin (Ig) synthesizing and secreting cells were enumerated in 11 DLE pts, 7 SCLE pts and 17 age, sex and race matched controls. Significant alterations in T cell subsets as determined by FACS analysis could not be detected except in SCLE pts (decreased number of OKT-8 cells). B cell numbers were significantly increased in the SCLE pts only. The percentage of Ig synthesizing B cells (cytoplasmic Ig containing cells) was increased in the DLE (3.2 ± 1.7%; $p = 0.002$) and SCLE (3.1 ± 2.4%; $p = 0.02$) groups when compared with the controls (1.5 ± 0.6%). In addition, the number of Ig secreting cells (hemolytic plaque forming cells) were elevated both in the DLE (1024 ± 494/10^6 B cells; $p = 0.01$) and SCLE (1116 ± 806/10^6 B cells; $p = 0.03$) pts compared to the controls (629 ± 253/10^6 B cells). The increases in Ig synthesizing and secreting cell numbers observed in the DLE pts could not be accounted for by underlying SLE activity, antimalarial treatment or the concurrence of the HLA-DR3 phenotype. These data demonstrate that evidence for polyclonal B cell activation similar to that which has been seen in SLE can also be found in LE pts whose clinical disease expression in limited to (DLE) or seen predominantely in (SCLE) the skin. It remains to be determined whether evidence of polyclonal B cell activation in a DLE pt. might represent a risk factor for the subsequent development of systemic disease activity.

4. Impaired Production of and Response to IL-2 in Spleen Cells in PUVA-Treated Mice. HIROYUKI OKAMOTO, TAKESHI HORIO, AND MASAHIRO TAKIGAWA*, Depts. of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, and *Hamamatsu Medical School, Hamamatsu, Japan.

It is well known that 8-methoxypsoralen plus long-wave ultraviolet light (PUVA) has several effects on immune responses. Interleukin-2 (IL-2) plays a central role in the T cell-dependent immune response. To determine whether in vivo PUVA treatment of mice could affect immune functions of spleen cells, we investigated the influence of PUVA on both production of and response to IL-2 in mouse spleen cells.

The shaved dorsal skin of female C3H and C57BL/6 mice was exposed to 2, 7, 20J/cm^2 UVA 45 min after topical application of 0.05% 8-methoxypsoralen. Culture supernatants from spleen cells stimulated with ConA (5 μg/ml) for 24 to 48 hr were assayed for IL-2 activity, using a mouse IL-2-dependent T cell line, CTLL-2. The response of ConA-stimulated spleen cells to exogeneous IL-2 was assessed by proliferation of the cells cultured with recombinant IL-2 for additional 48 hr. The production of IL-2 was depressed in mice with a single PUVA treatment, as compared with the untreated mice. The presence of skin lesions was not responsible for this inhibitory effect of PUVA on the production, because the phototoxic dose of PUVA was not necessary for obtaining this effect. The response to exogenous IL-2 was also impaired in PUVA-treated mice. IL-2 absorption studies indicated that the impaired response to IL-2 may result from the decreased

expression of IL-2 receptors on ConA-stimulated cells of the treated mice. These results suggest that immune dysfunction in PUVA-treated animals, previously reported, may be in part due to defective IL-2 production and response in spleen cells in these animals.

5. The Effect of Immunotherapeutic Drugs on Antibody Dependent Cellular Cytotoxicity (ADCC): Analysis of the Mechanism at a Single Cell Level Using Plaque Assay. SHINGO TSUDA, YUSUKE HANADA, HIROMI KIYONAGA, AND MIKIO ICHIKI, Dept. of Dermatology, Kurume University School of Medicine, Kurume, Japan.

The effect of immunotherapeutic drugs on ADCC was examined by a plaque assay, since ADCC was reported to be affected in some skin diseases. Monocyte-depleted effector lymphocytes (K-cell) from human peripheral blood, target sheep red blood cell (SRBC) and anti-SRBC IgG were added together to a poly-L-lysine treated microtestplate and, immediately after making monolayers, incubated at 37°C for 3 hours. The number and the size of hemolytic plaque (HP) of SRBC were examined in the presence of drug and/or culture medium. In the absence of drug, the percentage of plaque forming cell (%PFC) was 6.5 ± 0.3, and the size of HP was 53.4 ± 1.3 μm^2. The treatment of effector lymphocytes with recombinant interferon-αA(r-IFN-αA) resulted in the augmentation of activity of individual K-cell (2×10^3 U/ml of r-IFN-αA:HP 80.1 ± 3.4 μm^2, $p < 0.001$), but not in the increase of number of K-cell at any concentrations. Enhancement of both %PFC and HP were induced by unique immunopotentiators, Staphylococcal phage lysate (SPL) and Vaccinia virus products (NSP). (8×10^{-1} U/ml of SPL: %PFC 10.4 ± 0.6, $p < 0.01$, HP 65.8 ± 2.0 μm^2, $p < 0.001$; 100 μg/ml of NSP: % PFC 8.9 ± 1.1, $p < 0.05$, HP 67.2 ± 1.8 μm^2, $p < 0.001$). On the contrary, prednisolone (PS) suppressed the number of K-cell by a dose response (10 μg/ml of PS: %PFC 3.6 ± 0.9, $p < 0.01$). This suppression of K-cell by PS was observed only when the lymphocytes were preincubated with PS before mixing with anti-SRBC antibody. The average area of HP was significantly smaller (HP 43.6 ± 1.3 μm^2, $p < 0.001$). Thus, the plaque assay is vaulable to elucidate the effect of immunotherapeutic drugs on ADCC.

6. Monoclonal Antibody Detection of Nuclear Antigens in the Cytoplasm of Keratinizing Cells in the Skin and Mucous Membrane. S. NAKANO, K. FUKUYAMA, A. L. EPSTEIN, AND W. L. EPSTEIN, Dept. of Dermatology, University of California, San Francisco, CA, and Dept. of Pathology, University of Southern California, Los Angeles, CA.

Electron microscopic observations have suggested that nuclear components move into the cytoplasm during keratinization. In this study we examined the distribution of nuclear antigens in keratinocytes using monoclonal antibody probes (Ab) which identify nuclear proteins. Frozen sections from cheek skin and buccal mucosa of 2-day-old rats were reacted with Ab 58-15 and Ab 780-3 and stained with the Avidin-Biotin complex technique. Ab 58-15, which binds with 36 K protein, stained the nucleus strongly and the cytoplasm very faintly in basal cells. Nuclear staining was reduced and cytoplasmic staining became more dense in Malpighian cells. The cytoplasmic staining in the epidermis was diffuse and, for the most part, disappeared from the cells as they formed keratohyalin granules, whereas it was concentrated in the form of keratohyalin granules in mucosal epithelium. Ab 780-3, which reacts with both 105 K and 41 K proteins and concentrates in interchromatin granules, showed a speckled pattern confined to the nuclei of basal and lower Malpighian cells. As keratinization progressed in the epidermis, small granules staining similar to those in the nuclei appeared in the cytoplasm of some cells but no antigen was detected in cornified cells. On the other hand, in the mucosa the cytoplasm of all differentiated cells contained the stained granules in large numbers which were located mostly at the cell periphery. These findings suggest that nuclear proteins accumulate in the cytoplasm of keratinizing cells, particularly in the mucous membrane in defined compartments.

7. Host Immunologic Response in Penicillin/Ampicillin Drug Rash. K. A. FRITZ, A. BEYER, R. RAPINI, W. GOEGHEGAN, AND R. E. JORDON, Dept. of Dermatology, University of Texas Medical School, Houston, TX.

An evaluation of 4 immunologic parameters in 7 persons experiencing a non-urticarial allergic cutaneous reaction to penicillin (PCN) or ampicillin (AMP) is performed. Patient titers of IgG, M, A, E, αPCN & αAMP were assessed. Tissue samples were examined for 1) IgG, A, M, E by IF; 2) degree of endothelial swelling (ENDO↑) & amount of infiltrate (INF); 3) surface markers delineating T Cells (T) B cells (DR^+), monocyte (M) & Ts cells (Ts). They were studied a single time at differing intervals from the onset of the eruption: early (E) 1–2d, mid (M) 5–6d, late (L) 12–14 d. Parameters appear to vary based on study time relative to onset of eruption. Findings are shown below:

Pt	Histo		Cell Markers				Tissue Antibody				αPCN Serum				αAMP Serum			
#	Endo	Inf	T	DR+	M	Ts	IgG	IgA	IgM	IgE	IgG	IgA	IgM	IgE	IgG	IgA	IgM	IgE
(E)1	1+	1+	1+	1+	1+	1+	—	—	1+	—	Not Done				Not Done			
(E)2	3+	1+	1+	1+	0	0	–	+	–	–	—	2+	—	—	—	—	tr	—
(M)3	1+	2+	2+	3+	1+	2+	—	—	2+	—	2+	2+	—	—	tr	—	—	—
(M)4	3+	2+	2+	3+	1+	0	2+	—	2+	—	tr	2+	—	—	—	tr	—	—
(M)5	2+	2+	2+	3+	1+	1+	—	—	1+	—	1+	tr	tr	—	tr	1+	—	—
(L)6	1+	4+	2+	4+	0	1+	—	—	—	—	tr	3+	—	—	tr	tr	—	—
(L)7	3+	2+	—	1+	—	—	—	1+	—	—	Not Done				Not Done			

Endothelial swelling & perivascular infiltrate are consistently present, but vary in degree. T & B lymphocytes dominate the infiltrate with variable presence of monocytes and Ts subset. Tissue immunoglobulins are variable in degree & type present. Humoral response during the eruption episode is minimal & variable with high IgA αPCN predominating early. This type of drug eruption appears to be primarily a cellular phenomenon involving T & B lymphocytes & monocytes.

8. Paraproteinemia in Patients with Scleredema: Serum Effects on Skin Fibroblasts *In Vitro*. AKIHIDE OHTA, AARNE I. OIKARINEN, JAMES R. SEIBOLD, AND JOUNI UITTO, Harbor-UCLA Medical Center, Torrance, CA, University of Oulu, Oulu, Finland, and Rutgers Medical School, New Brunswick, NJ.

Four patients with scleredema and paraproteinemia were studied. Clinically, they presented with rapid onset of induration and nonpitting edema on the face and neck. The abnormal immunoglobulin in each case was different being either IgG-K, IgA-λ, IgA-K or IgM type. Histologically, the dermis was markedly thickened with excessive deposition of collagen. Sera from these patients were tested for their effects on connective tissue metabolism and cell proliferation in skin fibroblast cultures. Serum from one patient significantly stimulated collagen production, as measured by the synthesis of [^{3}H]hydroxyproline, in normal human skin fibroblast cultures, while serum from another patient stimulated collagen production in autologous cell culture. Additionally, serum from one patient stimulated [^{35}S]sulfate incorporation into sulfated macromolecules by normal fibroblasts. Serum from one patient significantly inhibited the cell proliferation, measured as [^{3}H] thymidine uptake, while serum from the remaining three patients demonstrated enhancement of DNA proliferation comparable to that noted with age- and sex-matched control sera. Thus, circulating serum factors, possibly related to the paraprotein, may enhance the synthesis of connective tissue macromolecules by skin fibroblasts *in vivo*, thus providing a mechanism for dermal fibrosis demonstrable both clinically and histopathologically in these patients.

9. Treatment of Urticaria Pigmentosum Lesions with Corticosteroids. JACK BARTON, ROBERT M. LAVKER, NORMAN SCHECHTER, AND GERALD LAZARUS, Dept. of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Recent studies in our laboratory revealed that chronic application of potent topical corticosteroids under occlusion to normal skin resulted in the loss of mast cells. We therefore investigated the effects of intralesional and topical steroids in urticaria pigmentosum (UP). 3 patients with UP had lesions injected with 10 mg (0.25 cc) of triamcinolone acetonide. After 4 weeks all patients showed a loss of Darier's sign and light microscopy and electron microscopy (TEM) revealed numerous vacuolated mast cells devoid of granules. A 90% reduction in tissue histamine was noted (1 patient) compared to untreated lesions. By 8 weeks there was a dramatic clearing of the plaques and a decrease in brown hyperpigmentation. By 12 weeks mast cells were undetectable by light and TEM. The injection sites remained dramatically improved for as long as 1 year, and histamine content was reduced 95% (1 patient), 48 weeks after injection.

Topical application of betamethasone dipropionate under occlusion to limited areas (8 hrs/day, 6 weeks) induced almost complete clearing of UP lesions in 6 patients (ages 1–58). Lesions treated with vehicle under occlusion demonstrated no change. There was a dramatic decrease in the number of mast cells in treated skin as assessed by

histological grading of toluidine blue stained 1 micron plastic sections. Decrease in hyperpigmentation was maximal 12 weeks after stopping treatment. At 6, 12, and 18 weeks post-treatment, lesions remained dramatically improved clinically.

These data indicate that steroids induce dramatic decreases in the excess number of normal appearing mast cells in UP, as well as inducing prolonged remission of UP lesions.

10. The Biological Activities of the Novel Topical Antipsoriatic Agent, 6-Chloro-1,4-diacetoxy-2,3-dimethoxynaphthalene (RS-43179). JOHN M. YOUNG, CHARLES J. BEDORD, D. V. K. MURTHY, MICHAEL C. VENUTI, GORDON H. JONES, LARRY DE YOUNG, WILLIAM A. AKERS, AND JUD R. SCHOLTZ, Syntex Research, Palo Alto, CA.

RS-43179, selected from a series of compounds, is being developed as a topical antipsoriatic agent. In contrast to some related compounds, it is colorless, non-staining, stable in a variety of formulations, consistently active in the Scholtz-Dumas psoriasis bioassay, and minimally irritating and/or sensitizing. Efficacy of 1% RS-43179 in gel and ointment applied without occlusion has been established in controlled clinical trials.

Application to normal mouse epidermis (up to 5 mg/day for 21 days) produced no irritation or sensitization; no changes in keratinocyte proliferation and differentiation were detectable by histology or by SDS-PAGE analysis of proteins. Topical RS-43179 produced dose-related inhibition of arachidonic acid-induced mouse ear edema (ED_{50} = 1mg/ear) but only modest inhibition of TPA-induced ear edema. *In vitro*, up to 200 μM RS-43179 was inactive or slightly stimulatory towards: mouse epidermal cyclooxygenase and 12-lipoxygenase; human platelet cyclooxygenase and 12-lipoxygenase; vesicular PG-synthetase; and soybean 15-lipoxygenase. However, RS-43179 inhibited the production of 5-lipoxygenase metabolites (IC_{50} ~ 15 μM) by ionophore stimulated human PMNs. 5-lipoxygenase isolated from RBL-1 cells was also inhibited (IC_{50} = 0.5 μM). Since others have reported elevated levels of lipoxygenase metabolites in psoriatic lesions, we propose that the antipsoriatic activity of RS-43179 results from highly selective inhibition of 5-lipoxygenase, and that RS-43179 either locally or systemically may be useful in other diseases showing elevated 5-lipoxygenase metabolites.

11. Oxygen Diffusion in Chronic Venous Ulceration. HANS H. MOOSA, VINCENT FALANGA, MICHEL S. MAKAROUN, ANDREW B. PEITZMAN, DAVID L. STEED, MARSHALL W. WEBSTER, AND WILLIAM H. EAGLSTEIN, Depts. of Surgery and Dermatology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

The etiology of venous ulcers has been attributed to a diffusion barrier to oxygen caused by fibrin deposition around dilated, proliferating capillaries. This diffusion barrier was studied in 10 patients with venous ulcers using the transcutaneous oxygen (TcPO2) monitor (TCM2, Radiometer, Copenhagen). TcPO2 sensors were placed adjacent to venous ulcers on 13 limbs and on the chest and foot of each patient. Readings were taken after a sensor temperature of 44°C was reached (10–15 minutes). TcPO2 values were markedly decreased in skin adjacent to the ulcers (9.92 ± 3.02 mmHg) compared to those of the chest (66.17 ± 2.45 mmHg) and foot (43.95 ± 3.91 mmHg). Administration of 100% oxygen for 10 minutes increased chest TcPO2 in 9/9 patients (144.75 ± 8.47 mmHg) and increased TcPO2 in skin around the ulcers in 8/9 patients (69.58 ± 17.47 mmHg). This study confirms the existence of a pathologic barrier to oxygen diffusion in patients with venous ulcers. However, oxygen delivery to the ulcerated areas as measured by transcutaneous oxygen monitoring can be augmented by administration of oxygen. Inhaled oxygen may provide an additional treatment modality in venous ulcers.

12. Development of a Stabilized Microemulsion 1% Anthralin Gel. LYNN A. DRAKE AND RICHARD WEHR, Dept. of Dermatology, Emory University, Atlanta, GA, and Bristol-Meyers Medical Department, Buffalo, NY.

We have developed a stable 1% anthralin (1,8-dehydroxy-9-(10H)-anthrocenone) molecule by utilizing an anionic emulsifier to produce a new microemulsion gel formulation. Anthralin is subject to degradation by oxygen, especially the hydroxide (OH^-) moiety, and typical emulsions (50% water) contain an abundance of (OH^-) ions. In an anhydrous base anthralin is relatively stable due to lack of (OH^-) ions. An effective formulation must release the drug and provide a mechanism for percutaneous penetration. The anionic emulsifier surrounds the anthralin with negative charges which repel the similarly negative charges of the (OH^-) ions. This prevents the oxidation and degradation of anthralin to the dimer form and anthraquinone. It also provides a high volatility co-efficient (Shima, et al) which facilitates absorption. Eighty (80) patients with side matched chronic plaque psoriasis were treated for six weeks with this 1% anthralin gel formulation on one side of the body and unmedicated vehicle control on the opposite side, in either a short contact (20 min) or long contact (8 hr) regimen. Weekly evaluations of scaling, erythema, plaque elevation and severity were performed and analysis of variant indicated that 1% anthralin gel and vehicle produced a significant reduction ($p \leq 0.0073$) in disease activity. There was no significant difference in efficacy with 1% anthralin gel between short and overnight regimens. There were 30% fewer reports of irritation in the short contact regimen. This new stabilized microemulsion 1% anthralin gel formulation provides a safe and effective short contact method for treating psoriasis.

13. Teichoic Acid Antibodies and Atopic Dermatitis. ANTHONY A. GASPARI AND ROBERT L. RIETSCHEL, Dept. of Dermatology, Emory University School of Medicine, Atlanta, GA.

This study examined the prevalence of serum antibodies to teichoic acid (TAA) (an antigenic component of the cell wall of *Staphylococcus aureus*) in a population of patients with atopic dermatitis (AD) (N = 52) and an age- and sex-matched control population (N = 51) (non-atopics without skin disease). Serum TAA were measured using an immunodiffusion assay.

There was a significant increase ($p < 0.01$) in the prevalence of low-titre (1:1 or 1:2) TAA in patients with AD (54%) compared with controls (24%). There was also an increased prevalence of high-titre (≥1:4) TAA in patients with AD (7%) compared with controls (0%) ($p < 0.05$).

The subjects' age had a definite influence on the prevalence of TAA in both atopics and controls. None of the atopics or controls less than 10 years old had TAA. The prevalence of TAA peaked in the 15- to 30-year-old age group, with 86% of subjects with AD and 40% of controls ($p < 0.01$) having TAA. Adult subjects over 30 years of age had a lower prevalence of TAA, with 45% of subjects with AD and 19% of controls ($p = 0.10$) positive for TAA.

The relationship of TAA to the atopic disease state was also examined. The presence and titres of TAA did not correlate with the severity of dermatitis, duration of atopy, asthma, rhinitis, hay fever, pruritus, or the number of flares in eczema. Serial TAA in four patients undergoing an exacerbation of their eczema demonstrated significant increases in titres that subsequently decreased after the resolution of their dermatitis, suggesting a relationship between TAA and an acute flare in dermatitis.

14. Increased DNA Synthesis of the Hair Follicular and Perifollicular Cells by Topical Minoxidil in the Macaque Scalp. HIDEO UNO, ADRIENNE CAPPAS, AND PAMELA BRIGHAM, Wisconsin Regional Primate Research Center and Dept. of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI.

Our previous study revealed that minoxidil accelerated cyclic progression of the resting (telogen and anagen I) vellus follicles in the bald scalp of stumptailed macaques and simultaneously induced enlargement of the size of regrowing (anagen II and III) and late anagen follicles. Using the same 12 animals being treated with daily topical application of minoxidil (5%) or vehicle (control) in the bald frontal scalp for 1-½ years, we studied autoradiographic localization of DNA synthesis in the hair follicles, epidermis, and glands of the frontal and occipital scalp. Biopsied skins were incubated with tissue culture medium (modified L-15, Gibco) containing 5 μCi of ^3H thymidin for 24 hours at 37°C. After washing with fresh medium for 10 minutes at 37°C and fixing with Bouin solution, thin sections (2 μ) of glycol-methacrylate-resin embedded tissues were coated with NTB^3 emulsion (Kodak Co.). Following exposure for 10 days at 4°C, the tissues were developed and counter-stained. In minoxidil-treated frontal skin, the number of labelled cells was markedly increased in primordial follicles (anagen II and III) and bulb and outer sheath of growing follicles compared with those follicles in control skin. Many labelled cells were also found in the perifollicular connective tissue sheath including the dermal papilla in treated skin. However, number and distribution of the labelled cells in the epidermis and sebaceous gland in the frontal scalp and the follicles in the occipital scalp showed no difference between minoxidil and vehicle treated cases. Minoxidil appears to stimulate cell proliferation specifically in the hair follicle as well as the perifollicular sheath.

15. Abnormal Follicular Keratinization (AFK): Prevention by all-trans-Retinoic Acid (RA). J. A. MEZICK, E. G. THORNE,

M. C. Bhatia, L. M. Shea, and R. J. Capetola, Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, NJ.

An essential factor in the pathogenesis of acne is AFK resulting in microcomedo formation. A useful model to study the induction of AFK involves the topical application of a comedogen to the external ear canal of the rabbit. The comedolytic activity of RA in this model system is well-documented; however, little attention has been directed to the prevention of comedogenicity by topical retinoids. The objective of this study was to evaluate the prevention of AFK by RA in comedogen-treated rabbit ears. RA at 0.1% in alcohol:propylene glycol (70:30, v/v) was applied topically (0.5 ml) to one external ear canal of the rabbit in the morning. Both ears were treated with comedogen (Acetulan) in the afternoon. Dosing was 5 consecutive days/week for 2 weeks. Three days after the last application, the animals were sacrificed, epidermis was separated from dermis for whole mount evaluation and sections of skin were prepared for histologic examination. Each whole mount was observed under a stereomicroscope utilizing a 2.25 cm^2 grid. The number of comedones in four quadrants was counted and totaled. RA produced a marked reduction (>70%) in the number of Acetulan-induced comedones. In summary, we describe a method to evaluate agents for prevention of comedogen-induced AFK. This model may identify clinically useful compounds to prevent comedogenicity.

16. Rapid Scanning Remittance Spectrophotometry in Measurements of Erythema, Pigmentation, and Edema. Steven L. Jacques, Margo Weischar, John A. Parrish, and Allan R. Oseroff, Dept. of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, MA.

A technique for rapid acquisition of the spectral remittance from human skin has been used *in vitro* and *in vivo* to quantify erythema and pigmentation, and to examine the effects of edema on skin optics. Using incident white light, spectral scans of backscattered radiation from 350–700 nm are acquired in 0.1 s by a Rofin spinning grating monochromator and fiber optic system. The characteristic remittance spectra for oxy- and deoxy-hemoglobin and melanin have been determined in normal (skin types I–VI) and vitiligo patients exposed to graded doses of UVB radiation. The remittance spectra for 24-hr erythema and 7-day delayed tanning were analyzed to quantify the dose dependency of these cutaneous responses. The linear extrapolations of the 24-hr erythema data to the minimal UVB dose required to produce inflammation suggest a "true" MED that is ¼–⅓ the clinical, visually determined threshold. The sensitivity of the rapid-scanning system is sufficient to monitor the deoxygenation of blood which pools in the superficial venous plexus of forearm skin during the first minute after the arm has been lowered below the heart. A 25–35% increase in remittance of 650–700 nm light has been observed in forearm *in vivo* 24 hr following exposure to UVB doses sufficient to produce edema. A study of the effects of water content on the long-wavelength remittance of dermis *in vitro* also indicates that edema can significantly increase the backscattered light. This suggests edema may alter the effective light dose reaching the vascular bed, an important consideration in both conventional phototherapy and laser therapies utilizing wavelengths within the "therapeutic window" (600–1100 nm).

17. Copper, Zinc-Superoxide Dismutase, Manganese-Superoxide Dismutase and Malondialdehyde in Atopic Dermatitis and Normal Human Skin. Hiroshi Ueda and Katsuhito Sugiura, Dept. of Dermatology, Fujitagakuen Health University School of Medicine, Topoake, Aichi, Japan.

It was well known that the exposure of human skin to ultra violet rays elevated the production of free radicals. Superoxide dismutase (SOD) has a potency to dismutate the superoxide radical, and finally inhibits the formation of malondialdehyde (MDA) derived from lipid hydroperoxide. In this study, we determined copper, zinc-superoxide dismutase (Cu,Zn-SOD), manganese-superoxide dismutase (Mn-SOD) by enzyme immunoassays and MDA in skin in order to elucidate the effect of sun exposure, aging and atopic dermatitis. Cu,Zn-SOD, Mn-SOD and MDA levels in exposure skin site were high rather than those in unexposure skin site, respectively. These were not significant difference in the contents of two SODs and MDA between in epidermis and in dermis, but the contents of two SODs and MDA in epidermis were somewhat higher than those in dermis. Two SODs and MDA levels in unexposure skin site with atopic dermatitis were high rather than in exposure skin site with atopic dermatitis, respectively. The MDA levels in skin was increased with the age after 30 years old. However, the contents of two SODs have no relation with the age. We conclude that enzyme immunoassays for two SODs are highly useful as a determination of two SODs in skin and that it provides a more sensitive index of skin diseases.

18. Non-invasive Quantification of Cutaneous Edema In Vivo with a 1 GHz Dielectric Probe. Steven L. Jacques and Daniel J. McAuliffe, Dept. of Dermatology, Harvard Medical School and Massachusetts General Hospital, Boston, MA.

A novel technique for non-invasive measurements of the water content of skin has been applied to the problem of quantification of edema *in vivo*. Three methods of experimentally inducing edema were studied: (1) topical application of arachidonic acid (2 mg in 20 μl of acetone) to a 1 cm diameter spot on the abdomen of hairless mice; (2) mild trauma by repeated slapping (40 slaps in 10 s) of the ventral forearm of human volunteers; and (3) exposure of the forearm to UVA and UVB radiation (3–4 × MED). The dielectric probe method records the phase shift of a 1 GHz reflectance measurement which varies in a linear manner with the water content of the tissue. The coefficient of variation is 1–3% for 5 readings on a given site. For calibration, *in vitro* skin samples were hydrated to various known water contents determined by weight and were measured with the dielectric probe. Normal baseline *in vivo* water contents (WC, g water/g total weight) are about 0.60–0.65; dermis soaked in saline reaches 0.86. The results are summarized: (1) arachidonic acid causes edema (38–45% increase in mouse skin WC, implies 3–4 fold swelling) which is evident in 5 min, reaches its maximum at 1 hr, and persists for 5 hr; normal and athymic mice yield similar responses; (2) slapping the human forearm causes an immediate erythema which disappears after 10 min; the edema reaches its maximum (7–11% increase in WC) at 20 min, and persists for 1 hr; (3) UVA radiation causes immediate onset of edema (20–30% increase in WC at 8 hr), in contrast to UVB radiation which causes a delayed edema (10–15% increase in WC at 1–2 days). We conclude that this method will aid the evaluation of tissue damage and the study of the inflammatory process.

19. Human Lymphoblastoid Alpha-Interferon in the Treatment of Refractory Condyloma Acuminata. E. A. Olsen, K. F. Trofatter, S. A. Gall, J. R. Medoff, C. E. Hughes, M. S. Weiner, and F. F. Kelly, Depts. of Dermatology and Obstetrics and Gynecology, Duke University, Durham, NC.

Eighteen males and 16 females with refractory condyloma acuminata (CA) were treated in a randomized double-blind trial of lymphoblastoid interferon (Wellferon) and ibuprofen (Motrin) 600 mg twice a day or placebo. Interferon, 3 megaunits (Mu)/m^2 was given IM daily for two weeks then three times a week (t.i.w.) for four weeks. Patients were followed closely for any adverse clinical or laboratory side effects and the CA were measured and photographed at regular intervals during the protocol. Nine of 34 patients had a complete response (CR) and eight of 34 patients had a partial response (PR: greater than 50% reduction in the baseline CA surface area). Sixty-eight percent of women versus 22% of men had a CR or PR. Four of the nine CR patients and two of the eight PR patients relapsed during a four week observation period off of any drugs. Patients with any CA were then randomized to surgical excision of the CA followed by interferon 3 Mu/m^2 t.i.w. for four weeks or the same dosage of interferon alone. Five of seven patients retreated with interferon alone had a PR and 1 of 7 patients had a CR. Four of six patients who had surgery followed by interferon remained clear. Fatigue and malaise were almost universal but were generally well tolerated. Mental status changes were noted. Mild leukopenia was common and elevated liver function tests were seen occasionally but resolved with interferon discontinuation. Intramuscular interferon, either alone or in combination with surgery, is an effective treatment for some cases of refractory CA. Etiologies for discrepancies in response to treatment, including papilloma viral typing by DNA probe analysis and HLA typing, are being performed.

20. Three Dimensional Reconstruction of Veil Cells Around Normal, Diabetic and Aged Dermal Microvessels. Irwin M. Braverman, Jane Sibley, and Agnes Keh-Yen, Dept. of Dermatology, Yale University School of Medicine, New Haven, CT.

The veil cells, broad fibroblastic-like cells which surround all the dermal microvessels as a sheath, are more numerous and larger around the markedly thickened vessels present in sun damaged, diabetic, and in some cases of aged buttock skin. In most aged persons the vessel walls are abnormally thin and the veil cells are scant and appear underdeveloped by electron microscopy. To learn more about their morphology and spatial relationship, the veil cells around normal, diabetic, and aged vessels were reconstructed in 3 dimensions by

computer from 120 to 140 serial (80–90 nm) ultrathin sections. The veil cells were also studied by light microscopy from additional 120–140 serial 1 μm sections. The normal vessels were surrounded by a single layer of veil cells which had a wrinkled and pleated surface. The diabetic vessels were surrounded by 3–6 layers of cytoplasm produced by increased numbers of hyperplastic veil cells. The veil cells around aged vessels had the same length as young and diabetic veil cells but were much narrower so that they did not cover the vessels completely. The diabetic veil cells had 2 to 4 broad cytoplasmic sheets extending from the central nuclear portion of the cell body. These sheets were frequently folded back on themselves, thus producing the layering of cytoplasm around diabetic vessels. Punch biopsies studied by in vitro autoradiography with tritiated proline and lysine disclosed that young, diabetic and aged veil cells contained comparable numbers of silver grains per unit area of cytoplasm. The abnormal thickness of the vascular wall produced by a belt of basement membrane material and the abnormal thinness of the vascular wall appear to be related to the degree of veil cell development and to the number of veil cells around the vessels rather than to any differences in their basic metabolic activity in these different states.

21. Percutaneous Penetration Enhancement of Solvent-Deposited Solids by the Volatile Solvent. C. G. TOBY MATHIAS, RICHARD H. GUY, ROBERT S. HINZ, AND HOWARD I. MAIBACH, Dept. of Dermatology and School of Pharmacy, University of California, San Francisco, CA.

To study the influence of volatile solvents on the percutaneous penetration of solvent-deposited solids, we examined the effect of reapplication of acetone on the *in vitro* absorption kinetics of benzoic acid (BA), nicotinamide (NA), and hydrocortisone (HC) through hairless mouse skin. BA and NA (1 $\mu mol/cm^2$) and HC (0.1 $\mu mol/cm^2$) were dissolved in acetone and deposited on the skin surface; the solvent evaporated rapidly. Diffusion cells were perfused at equal rates such that the first order rate constant characterizing the output of penetrant from the diffusion cells into a fraction collector was 1.0 hr^{-1}. After 6 hours, acetone was reapplied to the skin surface and again evaporated rapidly. The effect on absorption kinetics 2 hours after reapplication was compared to controls. After normalizing the data for variations in peak absorption rates, significant increases in absorption rates above controls were observed for NA and HC (one-tailed t test, $p < 0.0025$ and $p < 0.025$ respectively). Although no significant increase was observed for BA, the normal decline in absorption rate following the peak was visibly changed following reapplication of acetone. From these observations, we conclude that volatile solvents are not "innocent bystanders", and may directly influence the percutaneous penetration process. Possible explanations include subtle damage to the stratum corneum, "solvent drag", or enhanced solubilization of solid penetrant into the stratum corneum.

22. Histologic and Immunopathologic Profiles of Alopecia Totalis Patients Receiving Topical Minoxidil (1% and 5%). V. C. WEISS, H. UNO, C. M. BUYS, A. CAPPAS, AND D. P. WEST, Dept. of Dermatology, University of Illinois, Chicago, IL, and Wisconsin Regional Primate Research Center, Madison, WI.

Topical minoxidil induces a dose-dependent hair regrowth in alopecia totalis (AT). Scalp biopsies of 3 patients (1 complete, 1 partial, 1 nonresponder) with AT of 2 to 3 years duration were evaluated histologically by serial sectioning and immunopathologically with anti-Leu 4 (pan T cells), Leu 3a (helper), Leu 2a (suppressor), Leu M1 (monocyte), Leu 7 (natural killer), OKT6 (Langerhan's) and OKT9 (activated T) using an immunoperoxidase technique. Hair follicles pretreatment were small with 80% in late anagen. Degeneration of the bulb was least in the complete responder and greatest in the nonresponder where it was accompanied by lamellar proliferation of the perifollicular fibrous tissues. The effect of minoxidil on follicular regrowth was more prominent with 5% than with 1% therapy. The complete responder showed return to normal of hair follicle structure and size. The partial responder showed return to normal of hair follicle length but not structure. The nonresponder showed some increase in follicle length and no change in structure. Pretreatment perifollicular infiltrates stained with anti-Leu 4, Leu 3a, Leu 2a and OKT9. Responders initially had fewer Leu 3a positive cells. Post treatment the complete responder showed absence of Leu 3a and OKT9, decreased Leu 4 and Leu 2a; the partial responder showed increased Leu 3a, no change of Leu 4 and OKT9, decreased Leu 2a; the nonresponder showed no change except decreased Leu 2a. These data suggest that severe degenerative follicular changes and relatively greater numbers of helper (Leu 3a) cells in the perifollicular infiltrates may be associated with greater resistance of AT to treatment.

23. Percutaneous Absorption of Steroids in Man Following Repeated Administration. DANIEL A. W. BUCKS, HOWARD I. MAIBACH, AND RICHARD H. GUY, Depts. of Pharmacy, Pharmaceutical Chemistry, and Dermatology, University of California, San Francisco, CA.

Percutaneous absorption studies usually involve single administration of the compound under investigation despite the fact that repetitive application is often the relevant *in vivo* situation. In this study, the effect of daily repeated topical application on the percutaneous absorption of hydrocortisone, estradiol and testosterone has been investigated in normal male subjects (n = 5). Skin absorption was determined indirectly by radioactivity excreted into the urine following topical administration and correction of these values for incomplete renal elimination. Steroids were applied every 24 hours to the same site on the ventral forearm at 4 $\mu g/cm^2$ for 14 days. The first and eighth doses of steroid were radiolabeled with carbon-14. Effect of multiple application was assessed by a paired Student's t-test between the percutaneous absorption of the first dose and that of the second radiolabeled steroid dose for each subject.

Steroid	*% Dose Absorbed*
Hydrocortisone	2.6 ± 0.7 (1st ^{14}C), 3.4 ± 1.4 (2nd ^{14}C)
Estradiol	9.9 ± 2.3 (1st ^{14}C), 10.8 ± 4.7 (2nd ^{14}C)
Testosterone	22.1 ± 6.9 (1st ^{14}C), 20.2 ± 6.8 (2nd ^{14}C)

No significant difference ($p > 0.05$) was found between the two carbon-14 dosings for each of three steroids. These studies suggest that steroid skin penetration resulting from daily repeated application (at comparable dose levels) may be predicted reliably from the measurement of penetration after a single administration delivered at the same surface concentration. [Supported by N.I.O.S.H. grant OH-01830 to RHG.]

24. Mechanism of Action of Tap Water Iontophoresis for Hyperhidrosis and Simple Innovations for Improving Its Efficacy. E. A. TEMPLETON, F. SATO, AND K. SATO, Marshall Dermatology Research Laboratories, University of Iowa College of Medicine, Iowa City, IA.

Tap water iontophoresis is the only nonsurgical therapeutic modality presently available for treatment of palmar hyperhidrosis, however, its efficacy varies. Moreover, it is not known whether tap water is indeed superior to other vehicles and, if so, why. A small metal electrode (0.5 cm^2) lined by a soaked thin nylon mesh was applied under a constant pressure while the subjects were resting. Skin resistance measured after 5 min presoaking the skin was 55K ohm cm^2 for saline and 71K ohm cm^2 for distilled water. The resistance of the latter was increased to 100K ohm cm^2 when a very thin layer of silicone paste was applied to the test site. That this increase in resistance is not due to blockade of sweat pore is shown by the same number of patent sweat pores before and after silicone application. Soaking the palmar skin in saline or tap water for 15 min drastically but temporarily reduced the patent pore number, which was prevented by coating the skin with silicone paste. We then iontophoresed (0.8 mA for 30 min daily × 10 days) the fingertips of 6 subjects using (1) tap water, (2) tap water with silicone paste, and (3) saline. In all 6 subjects (2) gave the most anhidrosis, whereas (1) ≃ (3) in 3, (1) > (3) in 2, and (1) < (3) in one subject. Iontophoresis-induced anhidrotic palmar skin did not show sweating in response to intradermal injection of methacholine (MCH) but cannulated sweat glands isolated from a biopsy skin specimen of the anhidrotic skin all showed avid secretory response to MCH in vitro. We conclude that iontophoresis-induced anhidrosis is due to ductal blockade without damage to the sweat gland and that insulating the stratum corneum with a hydrophobic paste such as silicone paste enhances the efficacy of iontophoresis.

25. Interactions of *Propionibacterium* with Serum of Inflammatory Acne Patients. WEI-LI LEE AND ALAN R. SHALITA, Dept. of Dermatology, Downstate Medical Center (SUNY), Brooklyn, NY.

Serum factors involved in the in vitro interaction of two strains of propionibacteria with polymorphonuclear leukocytes (PMNs) were studied employing the technique of chemiluminescence (CL).

Peripheral PMNs, purified through Methocel-Isopaque, were mixed with *P. acnes* and *P. granulosum* opsonized with whole and heat-inactivated normal and acne patient serum. Luminol enhanced CL was

measured at 22°C in a scintillation counter using serum treated zymosan as a positive control.

Opsonization and compliment had no effect on the CL response of PMN to *P. granulosum.* At 72 min, CL with or without serum was 2×10^5 CPM compared to control (PMN + serum) of 1.5×10^3 CPM. In contrast, opsonization greatly enhanced the response of PMN to *P. acnes;* opsonized *P. acnes* elicited the same response as unopsonized *P. granulosum* (2×10^5 CPM) whereas unopsonized *P. acnes* elicited only a minimal response (5×10^3 CPM). In addition, complement was required for efficient opsonization of *P. acnes.*

Our data reveal that there are quantitative instead of qualitative differences in the opsonic activity between the control and patient sera. In addition, difficult CL kinetics were shown for two strains of propionibacteria. The degree of the CL response appeared to be independent of the titers of anti-*P. acnes* antibody as determined by agglutination. These results suggest that immunoglobulin may interact with propionibacteria independent of specific antibody binding sites. Our results also support the concept that *P. granulosum* is an effective stimulator of PMN oxidative metabolism and phagocytosis.

26. Cysteine Proteinase Inhibitor (CPI) in Chemically Induced Squamous Cell Carcinoma (SCC). W. S. COOK, JR., S. NAKANO, K. YABE, M. NAKAGAWA, J. H. EPSTEIN, AND K. FUKUYAMA, Dept. of Dermatology, University of California, San Francisco, CA.

Cysteine proteinases have been considered to be involved with tumor growth and metastasis. We report the intracellular localization and biochemical properties of a CPI as a tissue regulator for cysteine proteinases present in SCC, the most common invasive skin cancer. The SCCs were experimentally produced on hairless mouse skin by a single application of dimethylbenzanthracene (400 μg). A portion of the excised tissues from SCC and normal skin were frozen for cryostat sections. Localization of the inhibitor was examined by the Avidin-Biotin complex immunohistochemical technique using rabbit anti-CPI IgG. The remainder of each tissue was heated at 60°C for 30 sec and protein was extracted in 10 mM Tris-HCl, pH 8.0, containing 0.15 M NaCl. CPI was purified by fast protein liquid chromatography equipped with a Mono Q column. The antigen was detected throughout the cytoplasm of normal epidermis while the staining changed to the nuclei of the SCCs. The extracts from 2 out of 3 SCCs contained CPI specific activity (4.34 and 3.45 mU/mg protein) greater than normal skin (1.38 mU/mg protein). The extract from the other SCC was within the normal range (1.27 mU/mg protein). CPI from both SCCs and normal tissue was eluted out from the Mono Q column with 20 mM NaCl. The purified CPIs had M_r 12,500 judged by SDS-PAGE. These findings suggest that CPI activity is increased in SCC development without changing its biochemical properties. CPI may control tumor growth, invasion and metastasis in SCCs of the skin.

27. The BULT Melanoma: A New Spontaneous Transplantable Tumor in Mice. WALTER C. QUEVEDO, JR., JACOB DYCKMAN, RUTH HALABAN*, AND GISELA E. MOELLMAN*, Division of Biology and Medicine, Brown University, Providence, RI, and *Dept. of Dermatology, Yale University School of Medicine, New Haven, CT.

The BULT melanoma first appeared in 1982 as a small intense black nodule on the tail of an adult female mouse of the LT/Ch strain (Brown University). The tumor has been maintained by serial subcutaneous transplantation of small fragments to the flanks of LT/Ch mice where it grows faster in males. Histological examination of the residual original tumor indicates that it is dermal in location and consists of heavily melanized ovoid to fusiform cells. Some ovoid cells containing extremely dense masses of coarsely granular melanin resemble macrophages. The transplanted BULT melanoma is externally bosselated, uniformly black and firm. Light microscopy reveals melanin-containing ovoid to fusiform cells with some nuclear atypism. Some cells resemble densely melanized macrophages. In the electron microscope numerous round to elliptical melanosomes at all stages of development are found within the parenchymal cells. The formative melanosomes display a filamentous matrix. In addition to melanosomes freely distributed in the cytoplasm, the melanoma cells contain groups of melanosomes sequestered within autophagic vesicles. Metastatic cells derived from the transplanted tumor have been observed in the axillary lymph nodes and lungs of host mice. Culturing of a lymph node suspected of having metastases confirmed the presence of melanocytes within it.

28. "Mucinous Carcinoma of the Skin" Is a Tumor of Apocrine Type. KAN NIIZUMA, SATORU MACHIDA, AND HIKARU ETO*, Depts. of Dermatology, Tokai University School of Medicine, Isehara, and *Kitasato University School of Medicine, Sagamihara, Japan.

The mucinous carcinoma of the skin is said to be derived from sweat glands or from derivatives of the germ of this gland. However, no definite conclusion has been reached as to whether the tumor is apocrine or eccrine in origin. In the present study, one histologically typical case of mucinous carcinoma of the skin was examined by both light and electron microscopy. Thus, absolutely convincing new evidence was obtained which has been never reported so far.

In addition to the routine stains of formalin-fixed paraffin embedded tissue, PAS stain with and without diastase digestion, colloidal iron stain and alcian blue stain at pH 2.5 and pH 0.4 with and without hyaluronidase and sialidase digestion were performed. Immunohistochemical studies were done by using monoclonal antibodies EKH 5 and 6 which detect eccrine gland associated antigens. Enzyme histochemical studies with formalin-fixed and non-fixed cryostat sections were carried out. Enzymes examined were as follows: acid phosphatase, β—glucuronidase, succinic dehydrogenase, malate dehydrogenase, phosphorylase, glucose 6-phosphate dehydrogenase and non-specific esterase. Specimens for electron microscopy were prepared by routine conventional method and studied of the serial sections.

Both enzyme histochemical and immunohistochemical examinations gave an apocrine type reaction. Further, one of the most salient findings of the electron microscopic studies was non-keratinized intracytoplasmic cavity which was described by Ken Hashimoto as a distinctive morphological feature seen in intrafollicular apocrine duct in human embryonic process.

It was concluded that the "mucinous carcinoma of the skin" is not a tumor of eccrine type but that of apocrine type.

29. Diverse Expression of Gamma-Glutamyl Transpeptidase (GGT) in Normal and Neoplastic Epithelial Cells of Human Skin: GGT As a Differentiation Marker for Malignant and/or Premalignant Epithelial Tumors. MASAHITO CHIBA AND KOWICHI JIMBOW, Dept. of Dermatology, Sapporo, Japan.

Differentiation of benign and malignant lesions in epithelial tumors is often difficult. We have recently shown that GGT is a differentiation marker of epithelial cells for malignant transformation during experimental carcinogenesis. This study investigated the expression of GGT activities in benign and malignant epithelial tumors with comparison to adjacent normal skin. The tumors studied included squamous cell carcinoma (SCC), basal cell epithelioma (BCE), Bowen's disease, Paget's disease, solar keratosis, keratoacanthoma (KA), seborrheic keratosis and epidermal cyst. In normal skin, histochemical GGT activities were found only in secretory portions of eccrine and apocrine glands and inner root sheath of hair follicles whereas none of GGT activities were seen in normal epidermis. In malignant and premalignant tumors, all of SCC exhibited intensive GGT activities with regional differences in intensities. In Bowen's disease and solar keratosis, GGT activities were seen in focal areas where the cells with atypia were located. In Paget's disease, GGT activities were seen only in those large, round cells scattered in GGT negative epithelial cells. None of cases with BCE exhibited GGT activity. None of benign epithelial tumors of KA, seborrheic keratosis and epidermal cyst expressed GGT activities. Our study indicated the diverse expression of GGT activities in benign and malignant epithelial tumors of human skin, thus GGT being a potential differentiation marker useful for most of malignant epithelial tumors.

30. A New Antigen Expression System of Endothelial Cells in Benign and Malignant Vascular Tumors. Y. SUZUKI, U. SOOD, J. D. CRISSMAN, AND K. HASHIMOTO, Dept. of Dermatology, Wayne State University, Detroit, MI, and VAMC, Allen Park, MI.

Several vascular and lymphatic tissues were examined immunohistochmically in order to study the patterns of immunoreactivity of endothelial cell (EC) markers of normal, benign, proliferative vascular, and lymphatic tissues. The case were: 6 pyogenic granulomas, 1 malignant angioendothelioma, 2 lymphangiomas, and 5 specimens of normal skin. The examined EC markers were: blood group H antigen, HLA-A, B, C, DR, monocyte platelet antigen (M/P), type IV collagen, laminin and factor VIII. Expression of these markers was evaluated in 12 cases of breast tumors including skin metastasis in order to distinguish between intravascular and intralymphatic tumor growth. Notable differences in staining patterns of vessels were found. First, M/P is an antigen whose expression is limited to monocytes, platelets, blood capillaries, and hyperplastic Ecs. Second, HLA-A, B, C, type IV collagen and laminin remain intensely positive even when ECs become hyperplastic or proliferative, whereas HLA-DR and M/P staining becomes decreased or absent. Third, blood group H antigen is demon-

strable in malignant angioendothelioma and in Kaposi's sarcoma. Fourth, factor VIII was sometimes positive in lymphatics, but blood group H antigen, HLA-A, B, C, DR, and M/P were consistently negative in normal lymphatics and lymphangiomas. In contrast, type IV collagen and laminin were positive both in blood and lymphatic channels. Using morphological criteria alone, previous authors have detected blood vessel invasion in 35% of breast tumors. According to our immunohistochemical findings, 3 out of 12 (25%) of breast tumors have been found to exhibit tumor extension into blood vessels.

31. Ultrastructural and Immunocytochemical Differentiation of Trichilemmal Tumorigenesis in Man and Animal Models. MIZUHO INAZU AND YUTAKA MISHIMA, Dept. of Dermatology, Kobe University School of Medicine, Kobe, Japan.

The isthmus of the anagen follicle shows histologically characteristic findings such as trichilemmal keratinization without keratohyaline granules, intricate infoldings between keratinized and nonkeratinized cells, and numerous membrane coating granules (MCGs). These characteristics are useful for determining the site of oncogenesis. In this report, we describe cytopathological findings in tumors with trichilemmal differentiation observed in man and animal models. The animal model was a hairless strain of rats ("bald") which had tumors spontaneously; the tumors grew into dome-shaped masses (1.5–2.0 cm i.d.) within 2–3 months after they were first observed. The findings common to man and animal models are as follows.

By light microscopy, the tumor exhibits a multi-columnar proliferating pattern with a funnel-like keratinizing center and tumor cells showing sudden keratinization without keratohyaline granule layers. Cells of the columnar nests become more highly keratinized and more bulky toward the center. The cells surrounding the central part are rich in glycogen. Electron microscopically, the cytoplasm of prekeratinizing cells appears clear and contains various-shaped MCGs showing characteristic lamellar structure and acid phosphatase activity. The prekeratinizing interface shows interdigitations and honeycomb-like infoldings of a thickened cell membrane. Immunocytochemically, tumor cells have positive reactions with anti-nail keratin monoclonal antibody (Hori et al;., J Inv Derm 82:424, 1984) and commercially available anti-keratin antiserum.

32. Antibodies to Epidermal Cytoplasmic Antigens Following Treatment of Multiple Basal Cell Carcinomas. D. J. GOLDBERG, D. TORRES, AND J.-C. BYSTRYN, Depts. of Dermatology, NYU School of Medicine and Cornell School of Medicine, New York, NY.

Increased immune responses to tissue antigens have been reported following cryotherapy of non-cutaneous malignancies. To evaluate whether the treatment method of cutaneous malignancies affects antibody response to epidermal antigens, we tested the sera of 11 patients with three or more cryotherapy treated basal cell carcinomas (BCC), 14 patients with three or more BCC treated with electrodessication and currettage, and 12 control patients without skin cancer for antibodies to epidermal cytoplasmic antigens by indirect immunofluorescence.

We found that the incidence of upper epidermal cytoplasmic (U-CYT) antibodies in cryotherapy treated patients (82%, 9 of 11 pts) was much greater than that in patients treated by electrodessication and currettage (21%, 3 of 14 pts). This difference was statistically significant ($p < 0.01$). The incidence of U-CYT antibodies in control patients (25%) was similar to that in patients treated with electrodessication and currettage. General epidermal cytoplasmic (G-CYT) antibodies and basal cell cytoplasmic (BCL) antibodies were not seen in any patients.

These results demonstrate that the method of treating BCC has an influence on immune responses to skin. Cryotherapy, but not electrodessication and currettage, augments the immune response to some epidermal antigens. This finding suggests that if cryotherapy has a similar effect on immune responses to tumor associated antigens, this form of therapy could increase resistance to development of further BCC.

33. Malignancy Affects Response to Tunicamycin Oppositely for Epidermal Cells As Against Fibroblasts. SHU-JEN CHEN, JOANNE MILLER, AND MIRIAM M. BRYSK, Dept. of Dermatology, University of Texas Medical Branch, Galveston, TX.

We have confirmed the findings in the literature that tunicamycin is cytotoxic to transformed fibroblasts but not to their nontransformed kin. With two mouse epidermal cell lines of common origin, we observe a contrary pattern: The malignant (PAM-211) cells are more resistant to tunicamycin than their nonmalignant (LC-7) counterparts, as measured by growth and viability. The multiplication of the PAM-211 cells is insensitive to doses of 0.1 μg/ml or less, while that of the LC-7 is progressively affected at all dosages. In the incorporation of radioactive sugar precursors, we find the greatest inhibition caused by exposure to tunicamycin for mannose, less for glucosamine, still less for fucose. The incorporation of each sugar is reduced more for the nonmalignant cells, by a factor of two for fucose, not so dramatically for the other two. Morphological changes are minor (unlike for the fibroblasts); in particular, the desmosomal junctions are not affected. On stained polyacrylamide gels, bands near 80K and 100K are intensified by exposure to tunicamycin for both cell lines, and a band near 30K also increases for the nonmalignant cells. The changes in Con A binding glycoproteins are the same for both lines, with a 190K band sharply reduced and an 80K band enhanced; the most prominent band, at 150K, is unaltered (and may be desmosomal).

34. Correlation Between Heparan Sulfate Synthesis and Tumorigenicity Phenotype of Ultraviolet Light Induced Murine Cutaneous Fibrosarcomas. M. PIEPKORN, H. CARNEY, AND A. LINKER, Depts. of Medicine and Pathology, University of Utah and Veterans Administration Hospital, Salt Lake City, UT.

In the UVR murine tumor model, the basis for variation in tumorigenic potential of these cutaneous fibrosarcomas, and of lines cloned from tumors of "progressor" or "regressor" phenotype, remains unexplained. To correlate cell surface structure with tumor phenotype, we have studied glycosaminoglycan (GAG) synthesis of cell lines varying in tumorigenicity in syngeneic hosts. Following in vitro labelling of monolayers of cell lines with ^{35}S and ^{3}H-glucosamine, the hyaluronic acid and sulfated GAGs of cytoplasmic, cell membrane, pericellular matrix and substratum adhesion compartments were isolated by preparative chromatographic techniques and selective enzymatic digestion. No differences were found in GAG quantities or compartmental distributions between the cell lines. However, cell surface-associated heparan sulfate (HS) molecules synthesized by tumorigenic and nontumorigenic cell lines were different. 94% of this cell surface GAG from a progressor line cloned from the tumor RD 1024 was degradable by nitrous acid deamination compared with 83% from a regressor clone of the same tumor; thus, the progressor HS has a higher N-sulfate content. In addition, we found a 12% difference in N-sulfate content between the same regressor line and a progressor line cloned from a different primary tumor. Furthermore, the overall density of HS anionic charge, by DEAE-Sephadex elution, was greater in a newly-isolated progressor tumor compared with a new regressor tumor. We conclude that there are consistent differences in the HS molecules synthesized by UVR tumors of different tumorigenic potential. HS may therefore function in some manner in the expression of the malignant phenotype.

35. Thy-1+ Epidermal Cells Are Not Demonstrable by Anti-Thy-1 Antibody in Human and Rat Skin. DONALD A. CHAMBERS, RHONNA L. COHEN, AND JOHN M. CRAWFORD, Health Sciences Center, University of Illinois at Chicago, Chicago, IL.

The presence of Thy-1+ epidermal cells in murine epidermis has been reported in this and other laboratories. These studies were designed to extend these observations to human and rat epidermis. Epidermal sheets from both human and rat skin, obtained after incubation in .76% EDTA separating solution for 2 hr at 37°C, were stained for indirect immunofluorescence microscopy. For analysis of human Thy-1+ cells, epidermal sheets were treated with a monoclonal anti-human Thy-1 antibody (supplied by Prof. J. Fabre, Queen Victoria Hospital, Sussex, UK) which had been prepared against Thy-1 protein purified from human brain. To our knowledge, this antibody is the only specific antibody to human Thy-1 protein available. The developing antibody was an FITC labeled goat anti-mouse IgG. In parallel studies, rat epidermal sheets were treated with two different anti-Thy-1 antibodies previously shown to react with brain, nerve and bone marrow cells of the rat: monoclonal anti-mouse Thy-1.1 (New England Nuclear, Boston) and Ox7, a polyclonal mouse anti-rat Thy-1 antibody (Sera Lab, Sussex, UK). Both the anti-Thy-1.1 and Ox7 antibodies are directed against the same Thy-1 antigenic determinant.

Results of these experiments reveal that no dendritic or round Thy-1+ cells were demonstrable in either human or rat epidermis. Thus, although the presence of Thy-1 protein can be demonstrated in murine, rat and human neuronal tissue, the cellular equivalent(s) to the murine Thy-1+ epidermal cell remains elusive in human and rat epidermis.

36. Expression of the Leu-8$^+$ Phenotype by Leu-3$^+$ T Cells in Allergic Contact Dermatitis. E. A. ABEL, A. VOLTERRA, R. M.

ADAMS, B. J. NICKOLOFF, AND G. S. WOOD, Dept. of Dermatology, Stanford University Medical School, Stanford, CA.

The presence of Leu-3$^+$ T helper cells in the delayed hypersensitivity response to contact allergens has been previously documented by immunohistochemical techniques. Dissection of the T helper/inducer cells into phenotypically distinct subsets is now possible with the use of monoclonal antibody anti-Leu-8. The functional significance of the Leu-3$^+$,8$^+$ T cell subset lies in its stimulation of the cell mediated immune response, whereas the Leu-3$^+$,8$^+$ subset provides functional help for immunoglobulin synthesis.

Four skin biopsies were performed in 3 patients referred for evaluation and treatment of allergic contact dermatitis to the Stanford Dermatology Clinics. Simultaneous biopsies for immunophenotyping were obtained in one patient with Rhus dermatitis from two different lesional sites, consisting of an acute eczematous vesicular dermatitis and an urticarial plaque. Specimens in two additional patients were obtained from positive patch test sites at 48 hours to dichromates in one case and to thimerosal in the other. Immunophenotyping was performed using monoclonal antibodies to selected markers of T cell differentiation and function by an immunoperoxidase technique.

In all four cases a predominantly Leu-3$^+$,8$^+$ helper T cell subset was observed. Our findings in acute contact dermatitis and in the positive patch test sites to recall antigens correlate with the functional role of Leu-3$^+$,8$^+$ positive cells in their inducer function for cell mediated immunity. Further studies are in progress to investigate other types of cell mediated immune response including the induction phase of allergic contact hypersensitivity as in DNCB sensitization, as well as response to intracutaneous testing with common antigens.

37. Expression of Ia Antigen and Differentiation of Macrophages in Granulomatous Inflammation. An Immunohistochemical and Ultramicroscopic Study of Murine Leprosy. O. TANJI, S. IZAKI, H. SHIMODA, T. HIBINO, AND M. IZAKI, Dept. of Dermatology, Iwate Medical University School of Medicine, Morioka, Japan.

A suspension containing 10^7 Mycobacteria lepraemurium was injected s.c. into CBA ($H2^k$) and C57BL/6N ($H2^b$), as well as C57BL/6N (nu/+, and nu/nu) mice. Infected mice were challenged with the ultrasonically treated or untreated mycobacteria into the foot pads. Both subcutaneous murine lepromas and challenged foot pads removed after 48 hours were ultramicoscopically and immunohistochemically investigated with anti-IA^k and IA^b monoclonal antibodies, as well as anti-Thy1.2.

The challenged foot pads of C57BL/6N and C57BL/6N(nu/+) mice showed swelling reaction (>0.1 mm), and demonstrated infiltration of IA-positive and Thy1-positive cells, revealing delayed hypersensitivity for the mycobacteria. A hypersensitivity-type murine lepromas were developed in such animals. The granulomas consisted of differentiated macrophages to the secretory-type (30%), lymphocytes (35%), and fibroblasts (26%) in the peripheral area. This observation was correlated with immunohistochemical findings. IA-positive and Thy1-positive cells were demonstrated in the periphery. However, the central area consisting mainly of phagocytic macrophages (58%) showed neither IA- nor Thy1-positive cells. Furthermore, immunologically inactive-type murine lepromas in C57BL/6N (nu/nu) and congenitally susceptible CBA mice were shown to consist entirely of phagocytic macrophages (73%), and IA- and Thy1-positive cells were not found. These animals did not show foot pad swelling reaction.

A role of IA-positive cells was suggested for development of granulomatous hypersensitivity.

38. Phagocytic Activity of Langerhans Cell Granules. SHOZO TAKAHASHI, MASAAKI MOROHASHI, AND KEN HASHIMOTO, Depts. of Dermatology, Toyama Medical and Pharmaceutical University, Toyama, Japan, and Wayne State University School of Medicine, Detroit, MI.

Function of Langerhans cell (LC) granules has not fully been clarified at the level of ultrastructural analysis. Clathrin coat structures are closely involved in receptor-specific endocytosis and intracellular protein transport. In this study we intended to elucidate the phagocytic activity of LC granules and their association with clathrin coat structures. Horse radish peroxidase was used as a tracer. The epidermis of Guinea pig was separated with 0.2M EDTA in PBS and incubated in MEM containing 0.15% peroxidase. After incubation and rinse the tissue was fixed in 5% glutaraldehyde for 4 hours. The tissue was reacted with diaminobenzidine and hydrogen peroxide. After rinsing the tissue was processed for electron microscopy. Peroxidase-positive rod and round structures were observed in the cytoplasm with electron density. Some of them were recognized as LC granules. When the tissue was incubated in peroxidase for 30 minutes, peroxidase-positive LC granules were found near the cytomembrane. When incubation was performed for 60 to 120 minutes, peroxidase-positive LC granules connected with round structures were seen in the cytoplasm. Some LC granules near the cytomembrane had clathrin coat structures when the section was processed for routine electron microscopy. These findings suggest that LC granules have phagocytic activity and clathrin coat structures are related not only to receptor-specific endocytosis but also endocytosis of peroxidase.

39. Enhanced Plasminogen Activator Activity in Involved Epidermis of Hailey-Hailey Disease. PAMELA JENSEN, SHINJI MORIOKA, STUART LESSIN, AND GERALD LAZARUS, Dept. of Dermatology, University of Pennsylvania, Philadelphia, PA.

Benign chronic pemphigus (Hailey-Hailey disease) is an autosomal dominant genodermatosis characterized by acantholysis of the epidermis similar to pemphigus vulgaris. The protease plasminogen activator (PA), induced by pemphigus autoantibodies to epidermal cell surface molecules, appears to be a mediator of the cellular dyshesion characteristic of pemphigus vulgaris and foliaceous. We have investigated the hypothesis that increased PA, generated without involvement of autoantibody, may play a role in the generation of acantholytic lesions in Hailey-Hailey disease. Biopsies were taken from involved and noninvolved areas of 5 Hailey-Hailey patients. Epidermis was separated, freeze-thawed, homogenized, extracted in low salt buffer, and then extracted in 2M KSCN to recover PA. The specific activity of PA was calculated using DNA content of epidermis. In all cases, PA activity was from 5 to 100 times higher in involved epidermis than in noninvolved. PA activity in noninvolved epidermis was always in the normal control range. The activity of Cathepsin D, a lysosomal enzyme, was similar in involved and noninvolved epidermal extracts. To evaluate PA immunocytochemically, we incubated frozen sections from involved and noninvolved skin with antibody prepared against human urinary PA (urokinase). We observed specific staining for PA only in the involved skin of Hailey-Hailey patients. Staining was most intense in the nuclear region of the basal cells, although lighter staining was frequently observed throughout the involved epidermis. These findings support the hypothesis that enhanced PA may be involved in the induction of acantholysis present in Hailey-Hailey disease.

40. Prostaglandin D_2 and Prostaglandin D Synthetase in the Skin of Mast Cell Deficient Mice. KOUICHI IKAI AND TAKESHI HORIO, Dept. of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan.

Prostaglandin D_2 (PGD_2) is a major PG in mast cells and massively produced in patients with systemic mastocytosis. To evaluate the contribution of mast cells to tissue PGD_2 contents and PGD synthetase activity, we used mast cell deficient mice (W/W^v) and their congenic littermates (+/+) as controls. In this study, we compared PGD_2 contents and PGD synthetase activity in the skin of these two genotypes. The amounts of PGD_2 were determined by the radioimmunoassay specific for PGD_2. PGD_2 was found in the skin in concentrations of 14.9 ± 6.3 and 15.9 ± 3.0 ng/g wet weight in W/W^v and +/+ mice, respectively. Whereas, histamine contents in the skin were 0.23 ± 0.08 and 31.40 ± 4.92 μg/g wet weight in W/W^v and +/+ mice, respectively. On the other hand, three hours after irradiation with 500 mJ/cm^2 of ultraviolet light-B (UV-B), PGD_2 contents in the skin of +/+ mice were elevated three-fold in comparison with basal level before irradiation. However, such elevation was not observed in the skin of W/W^v mice. The activity of PGD synthetase was assayed in supernatant fraction (10,000 × g, 20 min) of homogenates of the skin. The skin of both genotypes showed 2.83/1.52 nmol/mg protein/min in the presence/absence of 1 mM glutathione, and no difference in the enzyme activity was found between W/W^v and +/+ mice.

These results suggest that mast cells contribute little to the total amount of PGD_2 and PGD synthetase activity in the skin of mice, at least under normal conditions, in spite of the absence of mast cells and the very low content of histamine in the skin of W/W^v mice. However, under stimulated conditions such as UV-B induced dermatitis, mast cells preferentially produce PG and play an important role in the development of inflammation.

41. Properties of iC3b Bearing IgG. KOHJI SAYAMA, YOSHIHARU MIKI, AND KOZO INOUE*, Dept. of Dermatology, University of Ehime, Ehime, and *Dept. of Bacteriology, Osaka University, Osaka, Japan.

C3b-IgG complexes are found in the sera of SLE, Sjögren's or Reiter's syndrome patients. The C3b binding to immune complexes is supposed to solubilize the complexes and to inhibit immune precipitation. This paper reports a further information of the mechanism.

Murine monoclonal anti-4-hydroxy-3-nitrophenacetyl (NP) IgG2a bound to NP-BSA-Sepharose was incubated with normal human serum at 37°C for 1 hour. C3b-IgG was eluted with NP-Gly, and purified with DE52 and Sephacryl S300. Affinity and avidity were measured by quenching method and by binding with NP-hexamethylene-diamine-Sepharose, respectively.

The bound C3 was iC3b on SDS-PAGE analysis, and iC3b-IgG reacted with both anti-human C3 and antimurine IgG on immunoelectrophoresis. Affinity of iC3b-IgG was reduced to ¼ to ⅕ of that of intact IgG, and avidity of iC3b-IgG was induced to 1/50 of that of intact IgG.

These findings indicated that the loss of the binding activity caused solubilization of immune complexes and inhibition of immune precipitation.

42. The Synthesis of Arachidonic Acid Metabolites by Human Keratinocytes. K. M. TRAMPOSCH, V. J. STEVENS, AND J. W. QUIGLEY, Dermatology Research, Bristol-Myers Pharmaceutical Research and Development Division, Buffalo, NY.

Cyclo-oxygenase (CO) and lipoxygenase (LPO) metabolites of arachidonic acid (AA) may be important mediators of skin inflammation. In addition, LPO products have been reported to be elevated in psoriasis which is a skin disease characterized by chronic inflammation and hyperproliferation. Animal and human epidermal homogenates or whole cells have the capability to convert AA to LPO metabolites. Grabbe et al. (Arch. Dermatol. Res. (1984) 276: 128–130) reported the synthesis of mono-hydroxy eicosatetraenoic (mono-HETEs) and leukotriene B_4 (LTB_4) by freshly isolated whole adult human keratinocytes. We report here the synthesis of CO and LPO products by freshly isolated neonatal keratinocytes. Human keratinocytes were harvested from neonatal foreskins by incubation overnight in 0.2% trypsin (22°C). Keratinocytes were incubated for 10 to 60 minutes at 37°C with 10 μM ^{14}C-AA and 2 μM of the Ca^{++} ionophore A23187. The reaction was stopped by the addition of methanol and the mixture was prepared for HPLC/online ^{14}C analysis. Neonatal keratinocytes produced 12- and 15-HETE and prostaglandin E_2 as major products in a time dependent manner. Unlike the previous work reported for adult keratinocytes, neonatal cells did not produce 5-LPO products such as 5-HETE or LTB_4. The synthesis of mono-HETEs were inhibited by standard LPO inhibitors which showed the following order of activity: 5, 8, 14, 14-eicosatetraenoic acid (ETYA) > nordihydroguaiaretic acid (NDGA) = BW755C > Benoxaprofen. As anticipated, indomethacin was ineffective at blocking HETE synthesis. These experiments suggest that the elevation of 12-HETE found in psoriatic lesions could be the result of keratinocyte synthesis and that inhibition of this enzyme could be a rational approach to the design of anti-psoriatic drugs.

43. Effect of Sulfonated Shell Oil Extracts (Ichthyol) on the Generation and the Biological Activity of Chemotactic Leukotrienes. BEATE M. CZARNETZKI, Dept. of Dermatology, University of Münster, Münster, F.R.G.

Chemotactic leukotrienes (CL) can be generated from human neutrophils and monocytes in vitro. CL have been demonstrated so far in several inflammatory dermatoses (psoriasis, atopic eczema). Ichthyol is an effective, noncarcinogenic therapeutic agent in leukocyte-dependent inflammatory processes. In the present investigation, the effect of ichthyol on the production and the biological effects of CL was investigated.

Human neutrophils and mononuclear cells were obtained by Ficoll/Hypaque separation and were incubated in buffer alone or with the calcium ionophore A 23187 for 15 min at 37°C. Cells were also preincubated for 30 min, 37°C in ichthyol at concentrations from 2 mg to 0.01 mg/ml and were then stimulated with the ionophore for another 15 min or were left in buffer alone. Supernatants were studied for CL during in vitro chemotaxis and for LTB_4 with a radioimmunoassay (Wellcome, England).

Ichthyol caused no significant release of CL or of LTB_4 by itself from either cell type, at any of the concentrations tested. It inhibited however the directed migration of leukocytes towards synthetic LTB_4 in a dose-dependent fashion at concentrations from 2—0.2 mg/ml. It also suppressed the release of chemotactic activity and of LTB_4 from both cell types at all test concentrations. These data suggest that ichthyol exerts potent anti-inflammatory effects by suppressing the production of LTB_4 from leukocytes and the chemotactic response of leukocytes towards LTB_4.

44. Comparable Production of Arachidonic Acid Metabolites by Epidermal Tissue and Keratinocyte Culture Preparations. JOHN FORTUNE, ELIZABETH DUELL, KATHI MADISON*, AND CYNTHIA MARCELO. Depts. of Dermatology, University of Michigan Medical School, Ann Arbor, MI, and *University of Iowa, Iowa City, IA.

Arachidonic acid metabolites (AA) are important mediators of inflammation in skin. To study the production of AA metabolites in whole skin, heat-separated epidermis and keratinocyte cultures from Balb/c neonatal mice, radioimmune assays (RIA) and high pressure liquid chromatographic (HPLC) analysis using a reverse phase system were done. Homogenates of whole skin, heat-separated epidermis and keratinocyte cultures were incubated with ^{14}C-AA for 30 min. Incubations were stopped with methanol and samples were extracted and partially purified prior to HPLC separation and spectral identification. Flow Radiomatic detector analysis of the HPLC effluent compared the ^{14}C-AA metabolites with authentic ^{3}H-labeled standards. Production of PGE_2 and $PGF_{2\alpha}$, and probably 15-HETE and 12-HETE was demonstrated in all three systems. Extremely large amounts of tissue and of epidermal cell cultures (60–70 × 10^6 cells) were needed to demonstrate small amounts of a metabolite tentatively identified as LTB_4 by Radiomatic analysis. Indomethacin decreased the production of prostaglandins by these preparations. Addition of 1 × 10^{-6} M TPA (tetradecanoyl phorbol acetate) to the keratinocyte cultures either short term (2h) or long term (6 days) increased the production of all metabolites. RIA of the medium collected from a 2 h incubation of intact cultures showed increased release of PGE_2 and $PGE_{2\alpha}$ by TPA treated cells. These studies showed that primary epidermal cell cultures have the same capacity for AA metabolism as intact epidermis and skin. Thus the interaction between AA metabolism and epidermal function can be studied using this culture system.

45. The Contribution of Cutaneous Neurons to the Inflammatory Response Evoked by Different Irritants. MAURICE E. LOOMANS AND GARY A. KERCKAERT, Miami Valley Laboratories, The Proctor & Gamble Company, Cincinnati, OH.

When capsaicin is administered to neonatal rodents, the peripheral sensory neurons degenerate. Therefore, clinically denervated animals can be produced and evaluated for their response to irritants. We determined that between day 10 and 15, neonatal mice are most susceptible to the neurotoxic effects of capsaicin administered at 50 mg/kg. When raised to adulthood, these animals had significantly raised threshold values in the hot plate test for analgesia.

At six weeks of age, the capsaicin- and vehicle-treated animals were tested for responsiveness to various irritating insults. Ear edema was determined gravimetrically after topical treatment with either croton oil, xylene, arachidonic acid, oxazalone after prior sensitization, or after a thermal burn. Of these irritants, only croton oil-induced swelling was significantly reduced in the capsaicin-treated group (55% inhibition). This suggests that the sensory neurons play a major role in inflammation produced by the active ingredients of croton oil. In support of this concept, we determined that the edematous response was inhibited 70–85% by capsaicin when administered either topically or systemically to adult animals. These results indicate that in certain types of cutaneous inflammation, the sensory neurons may have a significant effect.

46. Biological and Biochemical Characteristics of Eosinophil Chemotactic Factor Released from Keratinocytes *In Vivo* and *In Vitro*. K. TSUKAMOTO, T. KANZAKI, AND S. NISHIYAMA, Dept. of Dermatology, Kitasato University School of Medicine, Sagamihara, Japan.

Although some skin diseases are known to have eosinophil infiltrations in the epidermis, the precise mechanism is not elucidated yet. We revealed that human keratinocytes are able to synthesize an eosinophil chemotactic factor (E.C.F.).

A cell line was established from malignant trichilemmoma, which had many eosinophils among tumor cells histologically, in Eagle's M.E.M. with 10% F.C.S. The biological characteristics *in vitro* are as follows; 48 hrs in cell doubling time, 1% in plating efficiency, 6.5 × 10^4 cells/cm^2 in saturation density, and hypotetraploidy in karyotype. Of interest, tumors in nude mice again showed eosinophils among cells. Since cells were thought to have released E.C.F., the following studies were done for the confirmation of E.C.F. in the conditioned medium. The intradermal injections of media showed marked eosinophils in

guinea pigs *in vivo* (50 ± 12 cells/HPF; none in controls). Skin window technique showed similar results (10 ± 3; none in controls). E.C.F. was confirmed by the Boyden chamber method *in vitro* (74 ± 3 μm; control 49 ± 2 μm). E.C.F. was similarly produced by these cells in serum-free medium. This factor was found to be heat-labile and non-dialysable, suggesting that the factor is a protein in nature.

These results showed that human epidermal cells are able to produce E.C.F. other than E.T.A.F. This may explain the eosinophil infiltration in various skin diseases, such as pemphigus vegetans and incontinentia pigmenti.

47. Production of Arachidonic Acid Metabolites by Low Calcium Regulated Keratinocytes. J. A. FAIRLEY, E. A. DUELL, V. A. HOGAN, AND C. L. MARCELO, Dept. of Dermatology, University of Michigan Medical School, Ann Arbor, MI.

In vitro keratinocytes hyperproliferate when the Ca^{++} level of the culture medium is dropped to 0.02–0.09 mM and fail to form desmosomes or stratify when compared to cells grown at normal (1.2 mM) Ca^{++}. Since phospholipase A_2 is a Ca^{++} dependent enzyme we investigated the production of arachidonic acid (AA) metabolites by low-Ca^{++} regulated keratinocytes using radio immune assays (RIA) and reverse phase high pressure liquid chromatography (HPLC) with spectral analysis. Neonatal mouse keratinocytes were grown to day 4. The medium was removed and the cells washed with Ca^{++} free PBS. Test medium was placed over the cells for 2 hours. After the timed incubation the medium was removed and PGE_2, $PGF_{2\alpha}$, and LTB_4 were measured using RIA. The total DNA content of the cells was measured and the results expressed as pg/μg DNA/hour. Low Ca^{++} cells made 4.5 × the amount of PGE_2 as normal controls ($p = 0.002$) and 2.5 × the amount of $PGF_{2\alpha}$ ($p = 0.001$). LTB_4 was not detected by this method. Disrupted cells from low and normal Ca^{++} keratinocyte cultures were incubated with ^{14}C-AA for 30 minutes. The reaction was stopped with methanol and the lipid fraction was extracted and partially purified using a Seppak column. ^{3}H-labelled standards were added to the samples as tracers. Flow radiometric analysis of ^{14}C counts and spectral analysis demonstrated increased production of 15-HETE by the low Ca^{++} keratinocytes. RIA of the sample showed a 10 × increase in 15-HETE production by the low Ca^{++} cells. These studies showed that low Ca^{++} cells maintain intracellular Ca^{++} levels adequate for AA metabolism. Increased production of selected metabolites by low Ca^{++} cells is an interesting finding which requires further investigation.

48. The Investigation of the Action and Inhibition Spectrum of Solar Urticaria in Erythropoietic Protoporphyria. MASAKO TAKAHASHI, CHIDORI HIRABAYASHI, YOSHIKO KANNO, ITSURO MATSUO, AND MUNEO OHKIDO, Dept. of Dermatology, Tokai University School of Medicine, Isehara, Japan.

We investigated solar urticaria in 19 years old woman who was confirmed to be erythropoietic protoporphyria by the determination of protoporphyrin in erythrocyte (834 μg/100 mL packed cells) and feces (11345 μg/100g), and family history. The following studies were done: 1) Introduction of urticaria by lights. 2) Determination of action spectrum. 3) Investigation of the presence of spectrum which suppress the development of wheal by lights (Hasei, Arch Dermatol 118:346, 1982). The light sources used were natural light, the slide projector lamp (SPL) with 750W tungsten filament bulb and monochromatic light with xenon arc lamp. The results were as follows: 1) Urticaria was introduced by exposure to solar light, SPL and monochromatic rays. 2) The wavelength responsible for eliciting wheal exists in four ranges, 367 to 433, 488 to 554, 576 to 587 and 633 nm. These action spectra correspond to the absorption spectrum of protoporphyrin. The light intensity required to produce lesion were more than $3.3 J/cm^2$. 3) The longer spectrum of solar light or SPL than 700 or 660 nm through cut filter could suppress the urticarial reaction by following exposure to full spectrum of sunlight or SPL. Some monochromatic lights situated in wavelength which could not produce urticaria (600, 455 nm) inhibited reactions by some action spectrum (400, 410 and 500 nm) respectively. But other monochromatic rays (345, 350, 455, 466, 567, 567 nm) could not suppress wheal by 422, 400, 410, 411, 400, 500 nm respectively. Preirradiation of action spectrum (389, 422, 554, 587 nm) under threshold dose for urticaria were also found to inhibit urticarial reaction by 554, 587, 389 422 nm rays respectively.

49. Failure of Retinoic Acid to Inhibit UV-Induced Ornithine Decarboxylase Activity. L. H. KLIGMAN, Duhring Laboratories, University of Pennsylvania School of Medicine, Philadelphia, PA.

Ornithine decarboxylase (ODC) typically shows high activity in proliferating systems, viz embryonic tissue and regenerating rat liver. High levels are inducible in skin by such disparate agents as tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA), UV radiation, hair plucking and tape-stripping. Retinoids are well known inhibitors of induction by TPA. Repeated applications of all-trans retinoic acid (RA) in acetone have been reported to inhibit UVB-induced ODC in hairless mice. We wished to know whether RA in a cream vehicle would have the same effect. Groups of Skh-hairless-1 albino mice were irradiated with Westinghouse FS-20 lamps for one exposure of .045 J/cm^2 (≅3 human MED). Immediately post-irradiation, RA (100 μl) was applied to the dorsum in different concentrations (.001%, .002%, .02%), vehicles (cream and acetone) and on various time schedules (1–5 times). Sacrifice was by cervical dislocation 24 hrs later. Positive controls were treated with 34 nmol TPA. Epidermis was separated after immersion of dorsal skin in a 55°C water bath for 30 sec and two skins were pooled for each extract. Extracts representing 8–12 mice were assayed for each variable. Enhancement of UV-induced ODC activity was found with all concentrations and formulations of RA and with all schedules. UV alone induced a 70-fold stimulation while RA increased this by a factor of ~1.6. The degree of stimulation of ODC activity correlates with the promoting capacity of phorbol esters, hence ODC is considered a marker of tumor promotion. However, weak or nonpromoters such as mezerein induce epidermal ODC to the same extent as TPA. Since inducers of ODC activity, including RA, are hyperplasiogenic, it may be best to consider ODC stimulation a non-specific marker for proliferating cell populations.

50. Pheophorbide A Photoradiation Therapy for Chemically Induced Carcinoma of Rat Skin. NAONORI MAEDA, KAZUHIKO ICHIKAWA, TAISUKE KOBAYASHI, AND NOBUYUKI MIZUNO, Dept. of Dermatology, Nagoya City University Medical School, Nagoya, Japan.

Photoradiation therapy (PRT) with hematoporphyrin derivative (HpD) as a photosensitizer has already been used clinically for the treatment of cancer. But stronger photosensitizers are needed, because HpD-PRT is not sufficiently effective. A severe photosensitivity caused by pheophorbide a (PPa), one of the chlorophyll derivatives, was reported. We tried to utilize PPa for PRT of rat skin tumor induced by N-Methyl-N-Nitrosourea and obtained excellent results.

(1) Minimal necrosis light doses (MND) in animal skin of PPa and HpD were measured after ip injection of these substances (20 mg/kg), respectively. MND of HpD was 3.5 times as much as PPa. (2) PPa was retained in the tumor at a concentration 10 times higher (1.5 μg/g, wet wt) than in normal skin (0.15 μg/g, wet wt) 12–48 hrs after iv injection of 20 mg/kg. (3) Animals with tumors were irradiated (610–700 nm, $10.8 \times 10^6 J/m^2$) 24 hrs after iv injection of 20 mg/kg. This procedure was undertaken twice with 24 hrs interval, and the effect was estimated after 5 weeks. Macroscopically and microscopically, tumor size of treated group was 1/6 and 1/3.4 of untreated group respectively. (4) In the untreated group, malignant lesions accounted for 44% of all tumors, whereas only 17% of them were malignant in the treated group. Moreover, squamoid tumor was more sensitive than basaloid tumor to PPa-PRT among malignant lesions.

From the present results, it is concluded that PPa-PRT may be more effective than HpD-PRT, because of the stronger phototoxity and the longer light wavelength.

51. Skin Surface Conductance As a New Method for Assessment of Epidermal Damage by UVB. TAKAO ITO AND NOBUYUKI MIZUNO*, Biochemical Research Institute, Nippon Menard Cosmetic Co., Ltd., Ogaki, and * Dept. of Dermatology, Nagoya City University Medical School, Nagoya, Japan.

Although erythema has been utilized for the evaluation of sunscreen, it does not necessarily represent the tissue damage of skin by sunlight. For this purpose histologic observation is more exact, but there are many difficulties to apply it practically. A simple and reliable test has long been desired. We compared skin surface conductance, erythema, and histologic findings in the back skin of normal adult male after giving various doses ($0.6 \sim 2.4 \times 10^3 J/m^2$) of UVB with fluorescent sunlamp. Relative conductance was calculated from the conductance of irradiated and unirradiated skin measured with SKIN SURFACE HYDROMETER (IBS CO.) at 23°C, and 50% relative humidity. Because relative conductance became minimum at 6 days after irradiation of more than $2.4 \times 10^3 J/m^2$, measurement of relative conductance and biopsy were performed at 6 days. Minimal erythema dose (MED) was examined at 24 hrs after irradiation. 1) By irradiation of 1.2 and $1.8 \times 10^3 J/m^2$, relative conductances were more sensitive than erythema

($P < 0.05$). 2) By irradiation of 0.6×10^3 J/m^2, histologic findings were abnormal, whereas relative conductance and MED remained within the normal range.

From these results, relative conductance is found to be more sensitive and simple method for the determination of epidermal damage by UVB than MED, although histologic examination is the best.

52. Immuno-light and Electron Microscopic Study of DNase I Localization in the Nuclei of Epidermal Keratinocytes and Changes Following UVB Irradiation. YUMIKO YASUI, HIROYUKI SUZUKI, SHUN-ICHI BABA, TADASHI NOHARA, AND SADAO MORIOKA, Dept. of Dermatology, Niho University School of Medicine, Tokyo, Japan.

Histochemistry has shown that DNA within the nuclei of epidermal cells decreases during keratinization and is scattered throughout the karyoplasm after UVB irradiation. This study set out to investigate the localization of DNase I at various levels within the nuclei of keratinocytes and examine changes in DNase I distribution after UVB irradiation using both light and electron microscopy. Frozen sections of normal and irradiated (0.7–1.0 J/cm^2) skin from the ears of adult albino guinea pigs were fixed in 2.5% glutaraldehyde, stained with antiserum by the indirect enzyme immunohistochemical method, and prepared for light and electron microscopic observation. Thin sections were examined, and then observed a second time after double-staining with U and Pb. Reaction products were seen in various sized masses throughout the karyoplasm in basal and spinous cells, but in granular cells they accumulated in the center portion of the nuclei. One distinctive feature at the electron microscopic level was that reaction products were located in the electron luscent area within the nuclei. In addition, at 24–72 hr after UVB exposure, in more than 75% of epidermal cells the reaction products were scattered throughout the entire karyoplasm as small masses. These results suggest that DNase I is present at various levels in the euchromatin area within the nuclei of epidermal cells, and that after UVB irradiation the distribution of DNase I changes along with that of DNA.

53. Mechanism of Piroxicam-Induced Photosensitivity. I. E. KOCHEVAR, J. L. LAMMA*, D. J. MCAULIFFE, M. RODE, A. F. HOOD*, AND W. L. MORISON*, Depts. of Dermatology, Massachusetts General Hospital and Havard Medical School, Boston, MA, and * Johns Hopkins University Medical Institutions, Baltimore, MD.

Piroxicam, a nonsteroidal anti-inflammatory drug, in some patients causes photosensitivity that usually occurs within a few days of beginning the medication. This time course suggests a phototoxic reaction. The phototoxic potential for piroxicam was tested in normal volunteers after oral ingestion, in guinea pigs after i.p. administration, and in an *in vitro* lymphoblastoid assay. Piroxicam did not produce phototoxicity in any of these models in combination with UVB, UVA, or visible radiation. We next used lymphocytes *in vitro* to assay the phototoxicity of piroxicam and four of its metabolites: 5′-hydroxypiroxicam, N-methyl saccharin, saccharin, and 1,1-dioxo-2-methyl-4-hydroxy-2H-1,2-benzothiazine (DBZT). UVA exposures were between 1 and 40 J/cm^2. DBZT, a metabolite not usually detected in humans, was found to be phototoxic as measured by the decrease in the ability of cells to incorporate ^{3}H-thymidine after stimulation with PHA. A 50% decrease in cell viability occurred at 0.3 J/cm^2 UVA and 0.5 mM DBZT. All others tested were negative up to 40 J/cm^2. The same compounds plus a precursor to DBZT, 1,1-dioxo-2-methyl-3-carboxy-4-hydroxy-2H-1,2-benzothiazine (BZT), were tested for their ability to photosensitize membrane damage by measuring hemolysis of red blood cells. Only BZT and DBZT (both 0.9 mM) caused photohemolysis (50% lysis at 9.6 J/cm^2 UVA for BZT; 4.2 J/cm^2 for DBZT).

These results are consistent with a mechanism whereby piroxicam photosensitivity is due to a metabolite preferentially formed in affected patients. This would be a new and unique mechanism of drug photosensitivity.

54. Efficacy of Sunscreens Against Squamous Cell Carcinomas (SCC) in Hairless Mice. ISAAC WILLIS, JULIAN MENTER, AND MICHAEL FISHER, Dept. of Medicine/Dermatology, Morehouse School of Medicine, Atlanta, GA.

In an effort to determine to what extent sunscreens can prevent the development of skin cancer, we applied Homosalate, Pabanol, Coppertone 6, Coppertone 8, and Coppertone 15 (2 μl/cm^2) to 2 cm^2 sites on the backs of SK-1 hairless mice. In one set of experiments, groups of 10 mice were chronically irradiated starting with D = 9 J/cm^2 solar simulating radiation (290–400 nm), with incremental increases every 10 irradiation days for 10–14 weeks (Regimen I). A second set was irradiated in a similar manner, with $D_0 = 20$ J/cm^2 (Regimen II). Daily observations continued through three months post-irradiation revealed the following results: Regimen I mice exhibited benign hyperplasia which later regressed. No tumors were observed either immediately or 3 months post-irradiation (appropriate non-sunscreen controls produced tumors in roughly 80% of the mice at the end of irradiation, and in virtually all the mice 3 months post-irradiation). Regimen II mice showed no tumors at the end of irradiation, but 20% of the homosalate mice and 40% of the pabanol mice developed tumors or papillomas 3 months post-irradiation. These results indicate that sunscreens can retard, but not completely prevent SCC in a dose-dependent manner.

55. Mediators of Chemically Induced Skin Irritation. E. PATRICK AND A BURKHALTER, Dept. of Pharmacology, University of California, San Francisco, CA.

Studies describing components of inflammatory skin responses to six chemicals suggested that all chemicals do not produce irritation by the same mechanism. We have conducted studies comparing the mediators of responses to three of the irritants previously studied.

Female ICR mice 9–12 weeks of age were treated with agents known to block the effect of putative mediators and 5 mg methyl salicylate, 2 mg ethyl phenylpropiolate (EPP), or 0.05 mg croton oil was applied to one ear. Responses were quantified by measurement of ear thickness with a micrometer before application of the irritants and throughout the course of the responses. Response of treated and control groups was compared by t-test; a p-value of <0.05 was considered significant.

Indomethacin (1 mg/kg), an inhibitor of prostaglandin synthesis, reduced methyl salicylate and EPP responses and delayed the development of the croton oil response. Aprotinin, an inhibitor of kinin synthesis, significantly reduced responses to all three irritants. Pretreatment with 10 units cobra venom factor (CVF) depleted serum complement and reduced the EPP response. Pretreatment with 60 μg/kg mechlorethamine reduced white blood cells by 75% and reduced responses to croton oil and EPP but had no effect on the methyl salicylate response. Histamine antagonists cimetidine (1 mg/kg) and mepyramine maleate (1.5 mg/kg) significantly suppressed the response of EPP. The EPP response was also suppressed by methysergide maleate (1.5 mg/kg), an antagonist of 5 hydroxytryptamine. Antihistamines, methysergide, and CVF did not alter methyl salicylate and croton oil responses.

These results demonstrate differences in the mediators of irritant inflammation produced by these chemicals and confirm that chemicals produce irritant skin inflammation by multiple mechanisms.

56. Quantification of UV-B Dose Related Increase in Human Skin Blood Flow by Laser Doppler Velocimetry. ANTONY R. YOUNG, RICHARD H. GUY, AND HOWARD I. MAIBACH, Institute of Dermatology, London, U.K., and School of Pharmacy and Dept. of Dermatology, School of Medicine, University of California, San Francisco, CA.

Threshold skin sensitivity to ultraviolet radiation (UVR) is usually assessed visually in terms of the minimal erythema dose (MED). Intensity of response can be similarly assessed but with resultant subjective semi-quantitative data. Blood flow measurements with laser Doppler (LD) techniques allow objective measurements to be made. The mid-backs of human volunteers, with skin types ranging from I to VI, were exposed to a $\sqrt{2}$ incremental series of doses of UV-B (280–320 nm). Erythema was visually assessed 24 hours post-exposure and LD measurements of blood flow were made. The LD response offered no advantage when compared with the visual grading of a barely detectable erythema but enabled objective quantification of the vascular response. Irrespective of skin type, regression analyses showed a significant ($p < 0.05$) linear increase of blood flow with log dose over one order of magnitude. The photoprotective effect of melanin was quantitatively demonstrated to be of the order of a factor of 10 from the "right-shifted" dose-response curves obtained from pigmented skins. These studies show that LD techniques are an excellent non-invasive approach to the quantification of UVR induced changes of skin blood flow and the assessment of factors that may modify this response.

57. Characterization of Photosensitizing Effects by Topical Delivery of Alpha-Terthienyl and UVA. W. M. RAMPONE, J. L. MCCULLOUGH, G. D. WEINSTEIN, G. H. N. TOWERS†, AND M. W. BERNS*, Depts. of Dermatology and * Surgery, University of California at Irvine, Irvine, CA, and † Dept. of Botany, University of British Columbia, Vancouver, British Columbia, Canada.

Drugs now used for photochemotherapy are weak photosensitizers (tars), carcinogenic (psoralens) or produce prolonged photosensitivity (hematoporphyrin derivative (HPD)). α-Terthienyl (α-T), a phototoxic thiopene compound isolated from marigolds (Tagetes species), affects cell membranes and does not appear to induce cytogenetic damage. This study was undertaken to investigate topical delivery of α-T and characterize its cutaneous phototoxicity in combination with long wave ultraviolet (UVA) in comparison with locally (intradermal) administered α-T.

Percutaneous penetration (PC) of .1% and 1% α-T in a 2% Azone gel vehicle was studied in guinea pig skin *in vitro* and quantitated by UV fluorescence microscopy. Dose-dependent PC of epidermis, adnexae, and superficial dermis was demonstrated *in vitro*. 0.1% and 1% α-T in this same vehicle was applied topically *in vivo* and irradiated with 30 J/cm^2 UVA at intervals of 10 min–24h. Maximum sensitization was achieved with irradiation 1h following drug application. The clinical response was dose-dependent consisting of erythema, edema, crusting, erosion, and inhibition of hair growth and was observed 72h to 7d post-irradiation. Histopathologic changes paralleled macroscopic changes. α-T was also injected intradermally (5–500μg) and after 3h sites were irradiated with UVA (30 J/cm^2). A comparable dose-dependent response was observed. These results indicate that low-dose topical α-T in a non-irritating vehicle can rapidly produce cutaneous photosensitization. Topical α-T/UVA may provide a selective and safer alternative approach for the photochemotherapy of psoriasis and other cutaneous diseases.

58. Antigen-Specific Suppressor T Cells (Ts) Induced by Preirradiation of Photosensitizing Site to UVB. MASAHIRO TAKIGAWA, YOSHIKI TOKURA, MIZUHO YAMADA, AND YOSHIKI MIYACHI*, Depts. of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, and * Faculty of Medicine, Kyoto University, Kyoto, Japan.

We have reported that preirradiation of the photosensitizing site (ca 5 cm^2) with UVB (400 mJ/cm^2) renders mice unresponsive to a photochallenge reaction. The present study showed the generation of Ts specific for contact photosensitivity (CPS) to TCSA in the spleens of these unresponsive mice. Male BALB/c mice received UVB on the abdomen on day −2, and photosensitized with TCSA + black light (BL) at the same site on days 0 and 1. On day 5 spleen cells (SC; 10^8) from these mice were transferred to naive syngeneic mice that were subsequently photosensitized with TCSA + BL. Some SC were treated with monoclonal αThy-1.2 + C. All mice were photochallenged with TCSA + BL 5 days after the transfer. The ear swelling reaction at 24 hr was suppressed in mice that received untreated SC indicating the generation of Ts in SC. The post-proliferative response of draining lymph nodes (LN) was suppressed in Ts-received mice while cotransfer of photosensitized LN cells and Ts into naive mice did not inhibit a photochallenge reaction. Ts seemed to block the afferent limb of CPS. Antigen-specificity of Ts was shown by the failure to suppress the development of either CPS to other photoallergens or contact sensitivity to TCSA. Mice were photosensitized with TCSA + BL on days 0 and 1 while receiving i.v. supernatant (0.3 ml/mouse) from freeze-thawed SC and thymus cells from the unresponsive mice on days 0 to 5. Suppression of a photochallenge reaction in these mice indicated the presence of suppressive factor(s) in the sup. It was concluded that UVB-induced aberrant homeostasis of the skin caused a suppression of immune system that was associated with the generation of Ts and that such Ts inhibited CPS by liberating suppressive factor(s).

59. The Natural History of Contact Photosensitivity in BALB/c Mice. W. R. BROWN, G. M. SHIVJI, AND C. A. RAMSAY, Dept. of Medicine (Dermatology), University of Toronto, Toronto, Ontario, Canada.

In this study we examined the course and duration of contact photosensitivity (CPS) to tetrachlorsalicylanilide (TCSA) in BALB/c mice. Groups of 5 or more mice were photosensitized by applying 50 μl of 1% TCSA in acetone to clipped dorsal skin followed by 1.7 J/cm^2 of UVA on 2 consecutive days. The UVA source consisted of 2 GE F20T12BL UVA fluorescent lamps filtered by 3 mm of glass. Mice were challenged 1 week later with 10 μl of 0.5% TCSA in acetone on the ears followed by 1.7 J/cm^2 of UVA and the increase in ear thickness was measured after 24 hours. Challenge was performed at different times between 4 days and 9 weeks after sensitization. The challenge procedure in non sensitized animals produced an increase in ear thickness of 1.75 ± 0.89 (S.D.) × 10^{-3} cm. Ear thickness had increased by 4 days after sensitization (3.4 ± 2.7 × 10^{-3} cm) and reached a peak at 7 days (16.5 ± 5.2 × 10^{-3} cm). The peak response persisted for a further 7 days and then there was a gradual decline back to control levels at 7 weeks and beyond. The 9 week group of mice received a second sensitization. Challenge in this group one week later revealed a definite increase in ear thickness (9.6 ± 2.7 × 10^{-3} cm).

These results show that CPS in BALB/c mice wanes with time but in our study it persisted much longer than the 16 days previously reported, (Takigawa, M. & Miyachi, Y. (1982) J. Invest. Derm *79*, 108). The lack of response in mice first challenged 9 weeks after sensitization was not the result of complete tolerance because a second sensitization produced an increase in ear thickness on second challenge.

60. UVB/PUVA Induced Suppression of Human Natural Killer Activity Is Recovered by Superoxide Dismutase and/or Interleukin 2 In Vitro. KEN-ICHI TODA, YOSHIKI MIYACHI, AND SADAO IMAMURA, Dept. of Dermatology, Faculty of Medicine, Kyoto University, Kyoto, Japan.

Recent reports on ultraviolet (UV) induced suppression of natural killer (NK) activity in vitro prompted us to examine the effects of superoxide dismutase (SOD) and/or interleukin 2 (IL2) on this suppression. The peripheral blood mononuclear cells (PBMC) from healthy adults were separated by Ficoll-Hypaque gradient centrifugation and suspended in RPMI 1640 containing 10% fetal calf serum (FCS) at a concentration of 5 × 10^6/ml. 2 ml of this suspension with or without SOD (300, 1000IU/ml) amd/or IL2 (100IU/ml) were irradiated with 1 to 30 mJ/cm^2 of UVB (280–320 nm) or 0.1 to 5 J/cm^2 of UVA in the presence or absence of 8-methoxypsoralen (8 MOP, 0.1 μg/ml) at room temperature. After incubation at 37°C with 5% CO2 for 4 hrs, cells were washed and resuspended in RPMI 1640 containing 10% FCS at a concentration of 2 × 10^6/ml and used as effector cells. Non irradiated PBMC were used as controls. The NK activity was determined by 6-hr ^{51}Cr release assay, in which the K562 cells were used as target cells at the effector/target ratio 20:1. UVB/PUVA but not UVA radiation resulted in a dose dependent suppression of NK activity. Both SOD and IL2 were found to restore this suppressed NK activity. The addition of IL2 recovered the suppression more effectively than that of SOD. Combination treatment of UVB/PUVA irradiated PBMC with IL2 and SOD resulted in remarkable augmentation of the suppressed NK activity. These results suggest that administration of SOD and/or IL2 to the patient during phototherapy may be useful for restoring the suppressed NK activity, which is sometimes observed as adverse effects in the course of phototherapy.

61. The Effect of Photochemotherapy (PUVA) on the Development of Passive Cutaneous Anaphylaxis (PCA). RYOICHI KAMIDE, KIYOSHI YOKOI, AND SHUNICHI SAWADA, Dept. of Dermatology, Jikei University School of Medicine, Tokyo, Japan.

The effectiveness of PUVA in treating solar urticaria and mastocytosis has been reported, though the mode of action has not been clarified. In this study, we investigated the effect of PUVA on mast cell function, using rat PCA. 8-methoxypsoralen was applied to the depilated backs of Wister strain female rats, and they were then irradiated with 0.5 to 2 J/cm^2 of long-wave ultraviolet (UVA). As a control, half of the back was kept covered. Two hours, 2 days, and 5 days after PUVA treatment, PCA was elicited by intradermal injection of murine monoclonal antidinitrophenol (DNP) antibody, and subsequent intravenous injection of DNP-bovine serum albumin (BSA) mixed with ^{125}I-BSA in Evans blue dye solution. The magnitude of the PCA was measured by the exudation of ^{125}I-BSA in the antibody injected sites. Skin specimens were obtained from those sites and their radioactivity was counted in a gamma-counter. For comparison, compound 48/80 and 5-hydroxytryptamine (5-HT) were also injected intradermally. The vascular permeability changes induced by PCA and compound 48/80 were significantly suppressed in the PUVA-treated side at day 2. In contrast, PUVA did not alter the vascular permeability changes induced by intradermal injection of 5-HT. These results suggest that the PUVA induced suppression of mast cell degranulation may be involved in the therapeutic effect of PUVA.

62. Persistent Light Reaction in Experimental Animal Model. YOSHIO KATSUMURA, JUNKO TANAKA, SHINOBU KATO, TOSHIAKI KOBAYASHI, AND TAKEO MITSUI, Laboratory of Safety Evaluation, Shiseido Laboratories, Yokohama, Japan.

Persistent light reactions (PLR) due to photosensitizers have frequently been reported.

During the study of photoallergenicity of chemicals in guinea pigs, similar phenomena to those in humans were observed: 1) the animals

photo-induced with chemicals reacted to long wave length ultraviolet light (UV-A) in the absence of test materials at the challenge stage. 2) The sensitivity of animals to UV-A persisted as long as one year. 3) The minimum erythema doses of animals in treated group was less compared with those in control group.

Further investigation indicated that main factors influence the elicitation of PLR were the amount of Freund's Complete Adjuvant used to enhance the allergic response in animals, and UV-A dose. Based on these findings, we have established a PLR model in guinea pigs with high frequency.

63. Differential Effects of UV Radiation on Contact and Delayed Hypersensitivity Reactions to TNCB in the Mouse. MARGARET L. KRIPKE AND WARWICK L. MORISON, Dept. of Immunology, University of Texas System Cancer Center, Houston, TX, and Laboratory of Physical and Chemical Carcinogenesis, NCI-Frederick Cancer Research Facility, Frederick, MD.

Following epicutaneous sensitization of mice with trinitrochlorobenzene (TNCB), a delayed immune reaction can be elicited either by painting TNCB on the ears, in the classical contact hypersensitivity (CHS) reaction, or by injecting TNP-conjugated syngeneic spleen cells (TNP-SC) s.c. into the ears or the footpads. Subcutaneous injection of TNP-conjugated splenic adherent cells (TNP-SAC) also induces a delayed response that can be elicited by both procedures. Although these reactions are assumed to be equivalent and interchangeable, we find that they are affected differently by UV radiation. A single exposure of mice to UVB radiation from FS40 sunlamps at a dose of 49k J/m^2 suppressed the immune response to TNCB applied subsequently to unexposed skin. This was true whether the response was elicited by painting TNCB or by injecting TNP-SC into unexposed ears. However, immunization of the UV-irradiated mice with TNP-SAC obtained from normal syngeneic donors restored the response to TNP-SC, but did not restore the response elicited by painting TNCB on the ears. The response of UV-irradiated mice to TNCB also could not be restored by s.c. immunization with TNP-conjugated normal epidermal cells. This indicates that the inability of TNP-SAC to induce CHS in UV-irradiated mice was not due to the absence of an appropriate epidermal carrier antigen. These studies indicate that the immune reactions elicited by injection of TNP-SC and by painting of TNCB are not equivalent and that they are affected in different ways by UV irradiation.

12:00 PM–12:45 PM Presidential Ballroom

MONTAGNA LECTURESHIP AWARDEE

William Epstein, Presiding

Eugene Bauer

"Structural Integrity of the Skin: Insights Provided by Epidermolysis Bullosa"

12:45 CLOSING Presidential Ballroom

William Epstein and Yutaka Mishima, Presiding

INSTALLATION OF NEW OFFICERS (SID)

Papers Read by Title

Evaluation of the Split Skin Method for Differentiating Epidermolysis Bullosa Acquisita from Bullous Pemphigoid. P. Accetta, E. H. Beutner, V. Kumar, B. D. Wilson, and F. Helm, Buffalo, NY.

Dermal Absorption of ^{14}C-Nordihydroguaiaretic Acid (NDGA) in the Rat. Larry M. Allen, James A. Lugg, Jr., William J. Rees, Robert M. Parkhurst, and John H. Peters, Denver, CO, and Menlo Park, CA.

Outpatient 5-Fluorouracil Therapy for Resistant Psoriasis. Joseph C. Alper, Michael C. Weimann, Mary Kegel, Charles J. McDonald, and Paul Calabresi, Providence, RI.

Microbial Adherence to Various Human Regional Epithelial Cells. Raza Aly, Debra J. Bibel, Henry R. Shinefield, and Howard I. Maibach, San Francisco, CA.

Langerhans Cells and Basal Cell Carcinoma. Jag Bhawan, Donald J. Grande, and Brian Casey, Worcester, MA, and Boston, MA.

Ocular Pigment Cell Pathology in the Early Graying vi/vi Mouse Model for Vitiligo. Raymond E. Boissy, Gisela E. Moellmann, and Aaron B. Lerner, New Haven, CT.

Indirect Immunofluorescence and Lectin Binding Studies of Oral Lichen Planus and Response to Oral Isotretinoin Therapy. Charles Camisa, Richard Olsen, Brian Bowen, and Carl Allen, Columbus, OH.

Class II Antigen Expression in Normal Human Epidermis. M. M. Carr, P. A. Botham, E. McVittie, K. Guy, and J. A. A. Hunter, Edinburgh, U.K., and Macclesfield, U.K.

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